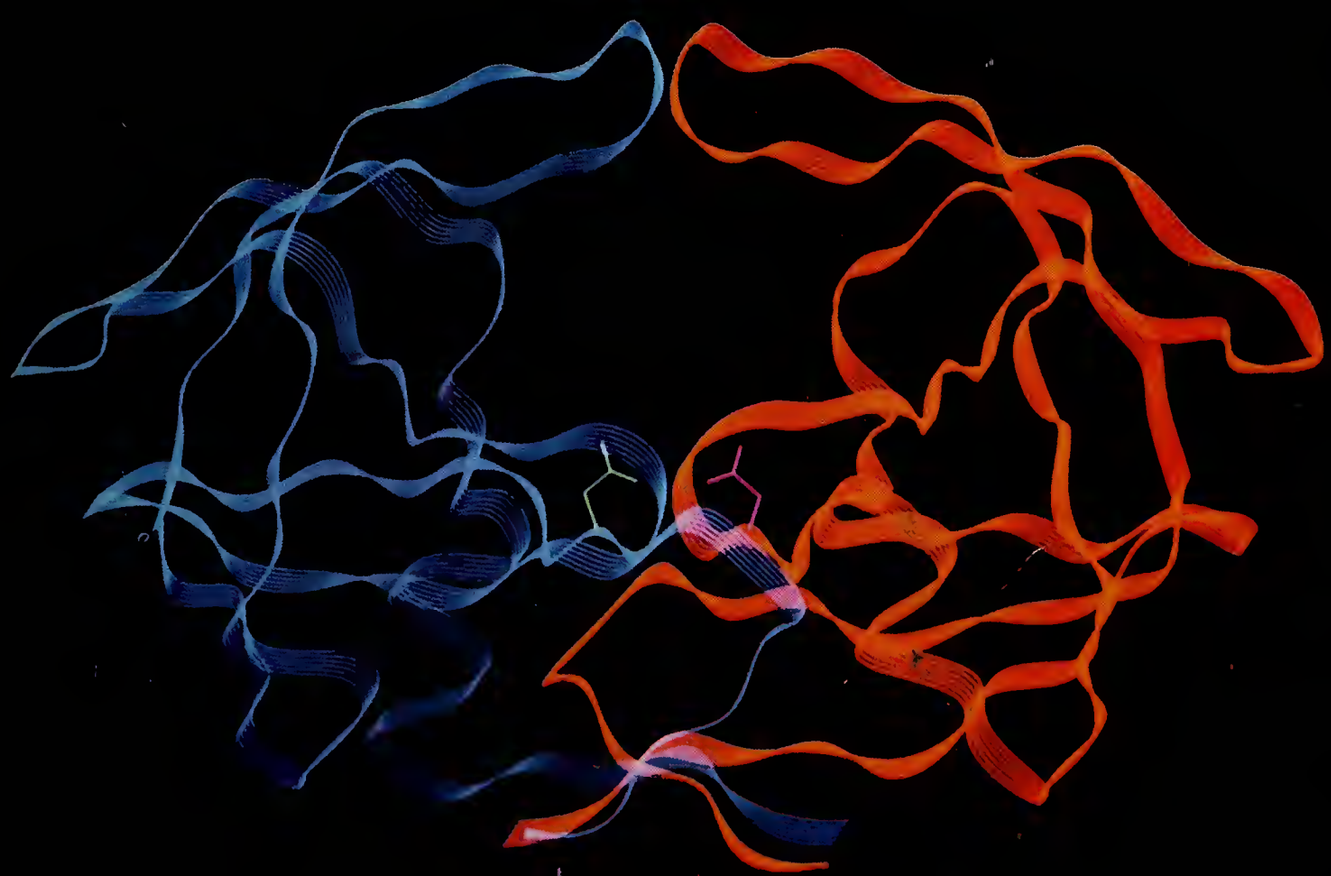
AIDS Research and Reference Reagent Program Catalog



U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

January 1990

Cover: Computer-generated molecular model of the crystal structure of HIV-1 protease. A member of the aspartic protease family, the enzyme is active as a dimer and is essential for viral replication.

Contributed by Dr. Alexander Wlodawer, Crystallography Laboratory, National Cancer Institute Frederick Cancer Research Facility.

Reference: Wlodawer, A., et al. *Science* **245**:616, 1989.

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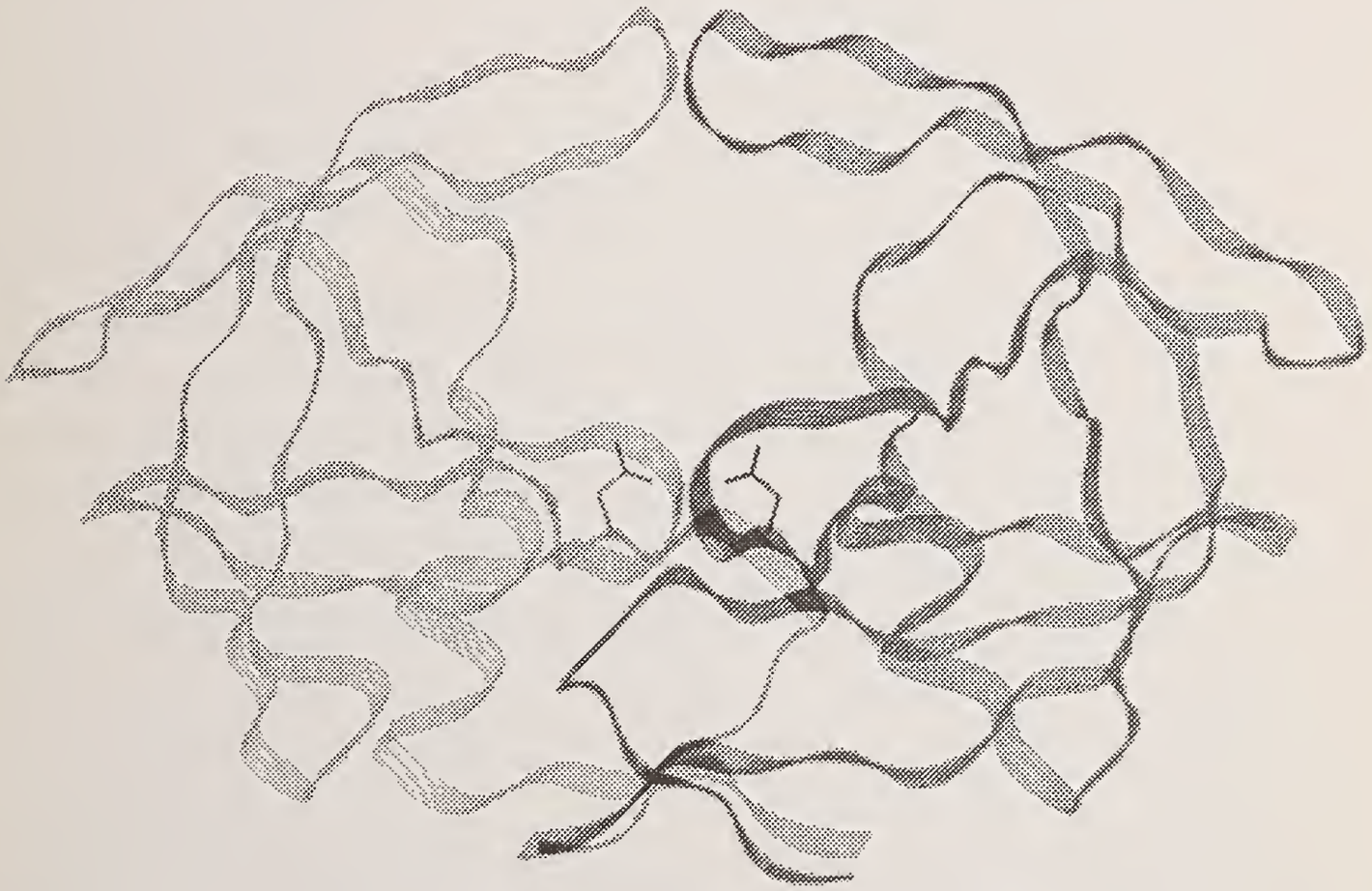

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A World Health Organization Collaborating Centre

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AIDS Research and Reference Reagent Program

New Policies and Procedures

The AIDS Research and Reference Reagent Program (Repository) was established by the National Institute of Allergy and Infectious Diseases (NIAID) to provide critical reagents for AIDS research to investigators throughout the world. The Repository is one of three World Health Organization (WHO) AIDS Reagent Centres. With the publication of the January 1990 catalog listing almost 50 new reagents, the Repository begins its third year of operation. We hope that Repository users will appreciate changes in the catalog. The entry format has been modified to provide a clearer description of items and the catalog contains a comprehensive reference bibliography to research reagents.

The most notable difference to users may be the simplified procedures for reagent requests. Previously, each request required multiple certifications by the requestor and by institutional officials. The new procedures require Laboratory Directors to complete an Annual Repository Registration Form which includes agreements, certifications, and shipping information. An *original signed* copy of the Annual Repository Registration Form is required. After the Registration is approved, requests require only a description of proposed reagent use and may be sent by FAX along with assurance that an original copy will be forthcoming.

Participation by for-profit organizations both as reagent donors and as recipients is essential to the success of the Repository. A major goal of AIDS research is to produce AIDS related drugs, vaccines and diagnostic tools. New language in agreements protects the commercial rights of donors of reagents. The previous absolute prohibition on commercial use of reagents was modified to allow commercial use of reagents, but only with *written permission and compensation* of the reagent donor(s) and notification of the Repository. Donors are under *no obligation* to grant permission for commercial use of reagents. To further protect donors, reagent recipients agree to notify the Repository and to negotiate in good faith with reagent donor(s) for compensation when a reagent is instrumental to a commercial discovery or development.

The Repository cannot assume liability for accidents or improper use of reagents after they have been delivered. An institutional indemnification agreement is required to protect the Repository and its contributors against such risks. Some institutions are unable to accept the indemnification agreement. We regret that biohazardous materials cannot be provided to research laboratories at those institutions.

Most of the reagents at the Repository are donated and the generosity of contributors is gratefully acknowledged. One reason donors provide reagents is to obtain additional information on the use of the reagents. Recipients should honor their commitment to describe the results of reagent use in semi-annual reports requested by the Repository. In addition, recipients should acknowledge in published works and disclosures the source of reagents as follows:

> *The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: Reagent _____ from Contributor_____, Reagent_____ _____ from Contributor _____, etc.*

All questions regarding reagents and procedures should be addressed to the Repository Principal Investigator, Susan Stern, PhD. Questions regarding policy, Division of AIDS, or NIAID should be addressed to the Pathogenesis Branch.

Gregory Milman, Chief
Linda M. Muul, Project Officer
Pathogenesis Branch
Division of AIDS, NIAID, NIH

Acknowledgment and List of Contributors

The AIDS Research and Reference Reagent Program solicits reagents from contributors, stores the reagents, and ships them directly to investigators upon approved request. On occasion small samples are provided by investigators and expanded by NIAID contractors. Information concerning the identification, purity, and activity of reagents is provided by the contributor who prepared the reagent.

The AIDS Research and Reference Reagent Program acknowledges with deep gratitude the generosity of those who contributed reagents prepared in their laboratories, answered questions, completed forms, and showed themselves willing to share material and information with the research community.

Rita Anand	Food and Drug Administration
Larry Arthur	Frederick Cancer Research Facility
Richard Axel	Columbia University
Luiz Barbosa	National Heart, Lung, and Blood Institute
Dani Bolognesi	Duke University Medical Center
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Irvin Chen	University of California
Edmond Cheng	E.I. DuPont de Nemours & Co., Incorporated
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Charles Flexner	National Institute of Allergy and Infectious Diseases
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Barton Haynes	Duke University Medical Center
Evan Hersh	Arizona Cancer Center
Michael Hershfield	Duke University Medical Center
James Hildreth	The Johns Hopkins University School of Medicine
James Hoxie	University of Pennsylvania Hospital
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Philip Johnson	National Institutes of Health
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George Lewis	University of Maryland
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Mikulas Popovic	New Mexico State University
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David Volsky	St. Luke's Roosevelt Hospital Center
Arthur Weiss	University of California at San Francisco
Alexander Wlodawer	Frederick Cancer Research Facility
Flossie Wong-Staal	National Cancer Institute
Janet Yamamoto	University of California at Davis
Paul Yoshihara	Epitope, Incorporated
Susan Zolla-Pazner	Veterans Administration Medical Center

CELL LINES

Reagent: 293
Catalog number: 103
Provided: 5×10^6 cells/vial.
Cell type: Human embryonic kidney cell.
Medium for propagation: DMEM, 90%; fetal bovine serum, 10%.
Freeze medium: Propagation medium, 90%; DMSO, 10%.
Growth characteristics: Cells grow in a monolayer and they double every 20 hours. Seeding ratio is 1:10.
Reverse transcriptase: Negative.
Special characteristics: Cells are sensitive to drying.
Contributor: Dr. Andrew Rice.
References: Rice, A.P. and Mathews, M.B. *Nature* 332:551, 1988.

Reagent: A3.01
Catalog number: 166
Provided: 2×10^6 cells/vial.
Cell type: Human T-cell line.
Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.
Freeze medium: Propagation medium, 90%; DMSO, 10%.
Growth characteristics: Cells grow in suspension.
Morphology: Mature lymphocytic.
Sterility: Negative for bacteria, mycoplasma, fungi, and protozoa.
Reverse transcriptase: Negative.
Special characteristics: These are a HAT-sensitive derivative of CEM and are suitable for human T-lymphocyte fusions. They are Leu-3^+, Leu-8^+, Leu-1^+, *tac*$^-$, transferrin receptor positive, sensitive to infection with LAV, and susceptible to cytopathic effects when infected.
Contributor: Dr. Thomas Folks.
References: Folks, T., et al. *Proc. Natl. Acad. Sci. (USA)* 82:4539, 1985.

Reagent:	**AA-2**
Catalog number:	135
Provided:	4×10^6 cells/vial.
Cell type:	Derived from WIL-2 human splenic EBV^+B lymphoblastoid line.
Medium for propagation:	RPMI 1640 supplemented with nonessential amino acids and 1 mM pyruvate, 90%; fetal bovine serum, 10%.
Freeze medium:	RPMI 1640, 67.5%; fetal calf serum, 10%; horse serum, 10%; DMSO, 12.5%.
Growth characteristics:	Cells grow in suspension.
Morphology:	Lymphoblast-like.
Sterility:	Negative for bacteria and mycoplasma.
Reverse transcriptase:	Negative.
Special characteristics:	This subclone of AA cells derived from the WIL-2 cell line expresses high levels of CD4 and binds more gp120 than other WIL-2 derivatives. The line is remarkably permissive for HIV-1 infection, extremely sensitive to virus cytopathic effects, and is useful for producing high titer virus stock.
Contributor:	Dr. Michael Hershfield.
References:	Chaffee, S., et al. *J. Exp. Med.* **168**:605, 1988.

Reagent:	**NIH-3T3 $T4^+$**
Catalog number:	156
Provided:	2×10^6 cells/vial.
Cell type:	Mouse fibroblast.
Medium for propagation:	DMEM, 90%; fetal bovine serum, 10%.
Freeze medium:	Propagation medium, 90%; DMSO, 10%.
Growth characteristics:	Similar to parent line.
Sterility:	Negative for bacteria, mycoplasma, fungi, and protozoa.
Reverse transcriptase:	Negative.
Special characteristics:	Even when rendered $CD4^+$ by retrovirus-mediated gene transfer, this mouse cell line is not susceptible to AIDS virus infection. It is used as a control in infectivity studies.
Contributor:	Dr. Richard Axel.
References:	Maddon, P.J., et al. *Cell* 47:333, 1986.

Reagent: CEM TK$^-$

Catalog number: 491

Provided: 5 x 10^6 cells/vial.

Cell type: Derivative of CCRF CEM, a human T-lymphoblast line.

Medium for propagation: RPMI 1640 with L-glutamine, 90%; fetal bovine serum, 10%; antibiotics.

Freeze medium: RPMI 1640, 70%; fetal bovine serum, 20%; and DMSO, 10%.

Growth characteristics: Doubling time is approximately 24 hours. Cells are grown in suspension at 0.2-2 x 10^6 cells/ml.

Morphology: Lymphoblast-like.

Special characteristics: This cell line has less than 1% of wild type thymidine kinase activity.

Contributor: Dr. Dennis A. Carson.

References: Personal communication.

Reagent: CEM-T4

Catalog number: 117

Provided: 2 x 10^6 cells/vial.

Cell type: Human T lymphoblastoid cell line.

Medium for propagation: MEM, 90%; fetal bovine serum, 10%; antibiotic free.

Freeze medium: Propagation medium, 95%; DMSO, 5%; antibiotic free.

Growth characteristics: Cells are grown in suspension. An inoculum of 10^5 cells/ml will increase four to five fold in 4-5 days when incubated at 37°C, providing pH is maintained at 7.0 and fresh medium is added every other day. Maintenance of the cell population at 10^6 cells/ml is optimal for growth.

Morphology: Lymphoblast-like.

Sterility: Negative for bacteria, mycoplasma, fungi, and protozoa.

Reverse transcriptase: Negative.

Special characteristics: CEM-T4 is a naturally isolated subclone of the CEM line with high levels of surface CD4 expression.

Contributor: Dr. J.P. Jacobs.

References: Foley, G.E., et al. *Cancer* 18:522, 1965.

Reagent:	**CHO ST4.2**
Catalog number:	501
Provided:	8×10^6 cells/vial.
Cell type:	CHO.
Medium for propagation:	Ham's F12 without hypoxanthine, with 0.3 μM methotrexate, and refiltered fetal bovine serum.
Freeze medium:	Propagation medium, 90%; DMSO, 10%.
Growth characteristics:	Medium growth rate. Maintain cells at about 2×10^6. Feed with medium at least every 3 days.
Morphology:	Flat adherent cell.
Special characteristics:	These cells secrete soluble CD4.
Contributor:	Dr. Dan Littman.
References:	Personal communication.

Reagent:	**CR10**
Catalog number:	391
Provided:	5×10^6 cells/vial.
Cell type:	Human T-lymphoid.
Medium for propagation:	RPMI 1640, 95%; fetal bovine serum, 5%.
Freeze medium:	RPMI 1640, 80%; fetal bovine serum, 10%; DMSO, 10%.
Growth characteristics:	Grows as a single cell suspension; doubling time 24 hours; should be maintained in the range of $0.2 - 1.5 \times 10^6$ cells/ml.
Morphology:	Round cells, somewhat larger than the parental CEM cells.
Special characteristics:	HIV-1 lysis-resistant subclone of CEM cells; CD4 receptor positive; suitable as a chronic carrier/producer of cytopathic HIVs; grows in HAT medium.
Contributor:	Dr. D.J. Volsky.
References:	Casareale, D., et al. *Virol.* 156:40, 1987.

Reagent: 174xCEM

Catalog number: 272

Provided: 2×10^6 cells/vial.

Cell type: Fusion product of human B cell line 721.174 and human T cell line CEM.

Medium for propagation: Iscove's Modified Dulbecco's Medium, 90%; fetal bovine serum, 10%. Also RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: Propagation medium, 90%; DMSO, 10%.

Growth characteristics: Cells grow in clumps. They express both T and B cell markers, including CD4.

Morphology: Hybrid cells are larger than CEM and appear oblong.

Sterility: Negative for bacteria and mycoplasma.

Special characteristics: This line has been found to be particularly useful for studies with SIV as it can be infected easily with that virus.

Contributor: Dr. Peter Cresswell.

References: Salter, R.D., Howell, D.N., and Cresswell, P. *Immunogenetics* **21**:235, 1985.

Reagent: CEM.NK^R

Catalog number: 458

Provided: 5×10^6 cells/vial.

Cell type: Human T-lymphoblastoid cell line.

Medium for propagation: Iscove's Modified Dulbecco's Medium, 95%; fetal bovine serum, 5%. Also RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: Propagation medium, 90%; DMSO, 10%.

Growth characteristics: Grows in suspension as single cells. Doubling time is 24 hours. Keep cells at $0.5\text{-}2.0 \times 10^6$ cells/ml.

Morphology: Round cells.

Sterility: Negative for bacteria.

Special characteristics: A variant of the CEM line resistant to natural killing; $CD4^+$, can be infected with HIV; useful in ADCC lysis studies.

Contributor: Dr. Peter Cresswell.

References: Howell, D.N., et al. *J. Immunol.* **134**:971, 1985.
Lyerly, H.K., et al. *AIDS Res. and Human Retroviruses* **3**:409, 1987.

Reagent:	**H9**
Catalog number:	87
Provided:	2×10^6 cells/vial.
Cell type:	Single cell clone derived from a specific HUT 78 cell line.
Medium for propagation:	RPMI 1640, 80%; fetal bovine serum, 20%.
Freeze medium:	RPMI 1640, 70%; fetal bovine serum, 20%; DMSO, 10%.
Growth characteristics:	This cell line was selected for high yield permissive growth with HIV-1.
Sterility:	The cloned cell population was extensively characterized to exclude the presence of adventitious viruses and mycoplasma and has been consistently negative in culture since 1984.
Contributor:	Dr. Robert Gallo.
References:	Mann, D., et al. *AIDS Research and Human Retrovirology* 5:253, 1989. Popovic, M., Read-Connole, E., and Gallo, R. *Lancet* ii:1472, 1984. Popovic, M., et al. *Science* 224:497, 1984.

NOTE: ***The use of the H9 cell line and other neoplastic T cell lines to produce HIV-1 is described in U.S. Patent 4,520,113.***

Reagent:	**HeLa-*tat*-III**
Catalog number:	502
Provided:	5×10^6 cells/vial.
Cell type:	Adherent fibroblast.
Medium for propagation:	DMEM supplemented with 250 ng/ml xanthine, 25 ng/ml mycophenolic acid, 10 ng/ml thymidine, and 60 ng/ml hypoxanthine, 93%; horse serum, 7%.
Freeze medium:	DMEM, 50%; fetal bovine serum, 40%, DMSO, 10%.
Viability:	80% upon thawing.
Growth characteristics:	These cells grow as a confluent culture and will grow in 3-4 days. Split 1:10.
Morphology:	Normal fibroblast appearance.
Reverse transcriptase:	None.
Special characteristics:	These cells produce the Tat protein from the HIV-1 provirus pHBC2.
Contributor:	Dr. William Haseltine and Dr. Ernest Terwilliger.
References:	Terwilliger, E., et al. *J. of Acq. Imm. Def.* 1:317, 1988.

Reagent: **HeLa-*env*-III**

Catalog number: 503

Provided: 5×10^6 cells/vial.

Cell type: Adherent fibroblast.

Medium for propagation: DMEM supplemented with 250 ng/ml xanthine, 25 ng/ml mycophenolic acid, 10 ng/ml thymidine, and 60 ng/ml hypoxanthine, 93%; horse serum, 7%.

Freeze medium: DMEM, 50%; fetal calf serum, 40%, DMSO, 10%.

Viability: 80% upon thawing.

Growth characteristics: These cells grow as a confluent culture and will grow in 3-4 days.

Morphology: Normal fibroblast appearance.

Reverse transcriptase: None.

Special characteristics: These cells produce the Tat, Rev, and Env proteins from the HIV-1 provirus pHBC2.

Contributor: Dr. William Haseltine and Dr. Ernest Terwilliger.

References: Terwilliger, E., et al. *J. of Acq. Imm. Def.* 1:317, 1988.

Reagent: **HeLa**

Catalog number: 153

Provided: 2×10^6 cells/vial.

Cell type: Human epithelial-like.

Medium for propagation: DMEM, 90%; newborn calf serum, 10%.

Freeze medium: Propagation medium, 95%; glycerol, 5%; antibiotic free.

Sterility: Negative for bacteria and mycoplasma.

Reverse transcriptase: Negative.

Special characteristics: This strain was used by the contributor to prepare HeLa $T4^+$ and HeLa $T8^+$.

Contributor: Dr. Richard Axel.

References: Scherer, W.F., Syverton, J.T., and Gey, G.O. *J. Exp. Med.* 97:695, 1953.

Reagent:	**HeLa CD4**
Catalog number:	459
Provided:	8×10^6 cells/vial.
Cell type:	Human cervical carcinoma.
Medium for propagation:	RPMI 1640, 90%; fetal bovine serum, 10%.
Freeze medium:	RPMI 1640, 80%; fetal bovine serum, 10%; DMSO, 10%.
Growth characteristics:	Rapid growth; repassage every 4-7 days splitting 1/10 to 1/50.
Morphology:	Variable, epithelial.
Sterility:	Negative for bacteria.
Special characteristics:	This cell expresses human CD4 protein on the cell surface and can be infected by most isolates of human immunodeficiency virus. Foci of HIV-1 infected cells can be detected by indirect immunoperoxidase or immunofluorescence using anti-HIV-1 serum or anti-HIV-1 monoclonal antibodies, or at a lower efficiency by syncytia formation. $CD4^+$ cells were selected by neomycin resistance after infection with a retroviral vector expressing CD4 and NeoR. Therefore, cells can be grown in the drug G418.
Contributor:	Dr. Bruce Chesebro.
References:	Chesebro, B. and Wehrly, K. *J. Virol.* 62:3779, 1988.
NOTE:	*NOTE: The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.*

Reagent:	**HeLa $T4^+$**
Catalog number:	154
Provided:	2×10^6 cells/vial.
Cell type:	Human epithelial-like.
Medium for propagation:	DMEM, 90%; newborn calf serum, 10%.
Freeze medium:	Propagation medium, 95%; glycerol, 5%; antibiotic free.
Sterility:	Negative for bacteria and mycoplasma.
Reverse transcriptase:	Negative.
Special characteristics:	Prior to retrovirus-mediated gene transfer with CD4 cDNA, these cells do not express surface CD4 and are not susceptible to AIDS virus infection. After transfection $CD4^+$ cells support infection by AIDS virus and the induction of syncytia.
Contributor:	Dr. Richard Axel.
References:	Maddon, P.J., et al. *Cell* 47:333, 1986.

Reagent: HeLa $T8^+$

Catalog number: 155

Provided: 2×10^6 cells/vial.

Cell type: Human epithelial-like.

Medium for propagation: DMEM, 90%; newborn calf serum, 10%.

Freeze medium: Propagation medium, 95%; glycerol, 5%; antibiotic free.

Sterility: Negative for bacteria and mycoplasma.

Reverse transcriptase: Negative.

Special characteristics: These cells are rendered $CD8^+$ by retrovirus-mediated gene transfer, but after this procedure they do not support infection by AIDS virus. They are used as a control for HeLa $T4^+$ infection studies.

Contributor: Dr. Richard Axel.

References: Maddon, P.J., et. al. *Cell* 47:333, 1986.

Reagent: HUT 78

Catalog number: 89

Provided: 2×10^6 cells/vial.

Cell type: Human cutaneous T cell lymphoma.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: Propagation medium, 90%; DMSO, 10%.

Growth characteristics: Split ratio is 1:10 and doubling time is about 26 hours.

Morphology: Mature lymphocytic cells; look convoluted.

Sterility: Negative for bacteria, mycoplasma, fungi, and protozoa.

Special characteristics: Cells are sIg and mIg negative, complement receptor negative and EBNA negative. They bear the IL-2 receptor and secrete IL-2 and migration inhibition factor. This line induces invasive tumors after intercranial injection.

Contributor: Dr. J.P. Jacobs and Microbiological Associates through Dr. David Madden, NIH.

References: Gazdar, A.F., et al. *Blood* 55:409, 1980.

Reagent:	**Jurkat Clone E6-1**
Catalog number:	177
Provided:	2 x 10^6 cells/vial.
Cell type:	Human T cell leukemia.
Medium for propagation:	RPMI 1640, 90%; fetal bovine serum, 10%.
Freeze medium:	Propagation medium, 90%; DMSO, 10%.
Growth characteristics:	Cells are passaged every 2-3 days. Maintain at between 10^5 and 10^6 cells/ml.
Morphology:	Lymphocytic.
Sterility:	Negative for bacteria, mycoplasma, fungi, and protozoa.
Reverse transcriptase:	Negative.
Special characteristics:	This clone of Jurkat-FHCRC (Dr. Kendall Smith, Dartmouth) produces large amounts of IL-2 after appropriate stimulation. Cells may be induced to secrete gamma interferon, and are $CD4^+$.
Contributor:	ATCC through Dr. Arthur Weiss.
References:	Weiss, A.L., Wiskocil, R.L., and Stobo, J.D. *J. Immunol.* 133:123, 1984.

Reagent:	**Molt 4 Clone 8**
Catalog number:	175
Provided:	2 x 10^6 cells/vial.
Medium for propagation:	RPMI 1640, 90%; fetal bovine serum, 10%,; antibiotics.
Growth characteristics:	Split twice a week 1:4 to 1:5.
Sterility:	Negative for bacteria, mycoplasma, fungi, and protozoa.
Contributor:	Dr. Ronald Desrosiers.
References:	Daniel, M.D., et al. *J. Virol.* **62**:4123, 1988. Kikukawa, R., et al. *J. Virol.* **57**:1159, 1986.

Reagent: **RHT-16**

Catalog number: 340

Provided: Amount will be provided on the data sheet sent with the shipment.

Cell type: Rabbit T cell.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%; 100 U/ml pen-strep; L-glutamine, 3%.

Freeze medium: RPMI 1640 with 20% fetal bovine serum, 50%; cryoprotective medium (MA Bioproducts, #12-132A), 50%.

Growth characteristics: Freeze medium should be washed out completely. Maintain cells at 0.1-2 x 10^6 cells/ml. Cells tend to grow in clumps. Retain about one-third of culture medium when splitting cells.

Morphology: Rounded; oval shaped.

Reverse transcriptase: Negative.

Special characteristics: Derived from rabbit PBL's infected *in vivo* with HTLV-I. Susceptible to HIV infection *in vitro*.

Contributor: Dr. Thomas Kindt.

References:
Truckenmiller, M.E., et al. Abst. #5185, *FASEB*, 1989.
Truckenmiller, M.E., et al. *Res. Immunol.* 140:527, 1989.

Reagent: **RL-5**

Catalog number: 341

Provided: 5 x 10^6 cells/vial.

Cell type: Rabbit T cell.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: RPMI 1640 with 20% fetal bovine serum, 50%; cryoprotective medium (MA products #12-132A), 50%.

Growth characteristics: Some clumping of cells, some aberration in morphology if pH of medium becomes acidic. Grows very slowly for the first 7-10 days when seeding from frozen cells.

Morphology: Rounded, oval, dumbbell shaped.

Reverse transcriptase: Negative.

Special characteristics: Derived from a *Herpesvirus ateles*-induced rabbit tumor from the inbred rabbit line B/J. Susceptible to HIV infection *in vitro*.

Contributor: Dr. Thomas Kindt.

References:
Kaschka-Dierich, C. et al. *J. Virol.* 44:295, 1982.
Kimball, E.S., Coligan, J.E., and Kindt, T.J. *Immunogenetics* 8:201, 1979.
Kulaga, H., et al. *Proc. Natl. Acad. Sci. (USA)* 85:4455, 1988.

Reagent:	**Sup-T1**
Catalog number:	100
Provided:	2×10^6 cells/vial.
Cell type:	Non-Hodgkin's T cell lymphoma.
Medium for propagation:	RPMI 1640, 90%; fetal bovine serum, 10%.
Freeze medium:	Propagation medium, 90%; DMSO, 10%.
Growth characteristics:	Seeding ratio is 1:10 to 1:20. Passage when number exceeds 5×10^5 cells/ml.
Morphology:	Mature lymphocytic.
Sterility:	Negative for bacteria, mycoplasma, fungi, and protozoa.
Reverse transcriptase:	Negative.
Special characteristics:	Cells are TdT positive, CALLA negative, and DR negative. They express pan T antigens, high levels of surface CD4, and lack sheep erythrocyte receptors.
Contributor:	Dr. James Hoxie.
References:	Smith, S.D., et al. *Cancer Research* 44:5657, 1984.

Reagent:	**VB**
Catalog number:	150
Provided:	2×10^6 cells/vial.
Cell type:	Thymocyte-like leukemia cells.
Medium for propagation:	Iscove's Modified Dulbecco's Medium, 90%; fetal bovine serum, 10%.
Freeze medium:	Propagation medium, 90%; DMSO, 10%.
Growth characteristics:	Cells are slow growing and form clusters and clumps. Cells should be passaged at 1:10 every 4-5 days.
Morphology:	Lymphocyte-like.
Sterility:	Negative for bacteria, mycoplasma, fungi, protozoa, and viruses.
Reverse transcriptase:	Negative.
Special characteristics:	Infection by HTLV-III or LAV results in a burst of virus production accompanied by a degree of cytopathic effect depending on the virus isolate.
Contributor:	Dr. Edgar Engleman.
References:	Lifson, J.D., et. al. *Science* 232:1123, 1986.

Reagent: X50-7

Catalog number: 498

Provided: Amount will be provided on the data sheet sent with the shipment.

Cell type: EBV transformed B-cell.

Medium for propagation: RPMI 1640, 92%; fetal bovine serum, 8%.

Freeze medium: Fetal bovine serum, 90%; DMSO, 10%.

Growth characteristics: Cells grow in single cell suspension with some clumping.

Special characteristics: This line is $CD4^+$ and capable of being infected with several HIV strains. The cells are capable of distinguishing HIV strains which undergo lytic, abortive, or noncytopathic persistent infection.

Contributor: Dr. George Miller.

References: Dahl, K., Martin, K. and Miller, G. *J. Virol.* **61**:1602, 1987.

Monoclonal Antibody Secreting Cell Lines

Reagent: Chessie 8

Catalog number: 526

Provided: Amount will be provided on the data sheet sent with the shipment.

Cell type: Mouse splenocyte/P3X63 Ag8.653 hybridomas.

Medium for propagation: RPMI 1640 supplemented with 50 μM β-mercaptoethanol, 2000 U/ml penicillin, 200 μg/ml streptomycin, 90%; fetal bovine serum, 10%.

Freeze medium: Fetal bovine serum, 90%; DMSO, 10%.

Growth characteristics: Cells grow well either in tissue culture or as ascites in incomplete Freund's adjuvant primed Balb/c mice.

Special characteristics: Chessie 8 produces a monoclonal antibody of isotype IgG_1. This antibody reacts with gp160 and is specific for gp41 as determined by ELISA and Western blot. Chessie 8 recognizes epitopes which survive alkylation and reduction. Antibody reactivities have been tested with $HIV\text{-}III_B$ isolates and its cloned *env* gene products.

Contributor: Dr. George K. Lewis.

References: Manuscript in preparation.

Reagent:	**Chessie 13**
Catalog number:	527
Provided:	Amount will be provided on the data sheet sent with the shipment.
Cell type:	Mouse splenocyte/P3X63 Ag8.653 hybridomas.
Medium for propagation:	RPMI 1640 supplemented with 50 μM β-mercaptoethanol, 2000 U/ml penicillin, 200 μg/ml streptomycin, 90%; fetal bovine serum, 10%.
Freeze medium:	Fetal bovine serum, 90%; DMSO, 10%.
Growth characteristics:	Cells grow well either in tissue culture or as ascites in incomplete Freund's adjuvant primed Balb/c mice.
Special characteristics:	Chessie 13 produces a monoclonal antibody of isotype IgG_1. This antibody reacts with gp160 and is specific for gp120 as determined by ELISA and Western blot. Chessie 13 recognizes epitopes which survive alkylation and reduction. Antibody reactivities have been tested using the $HIV\text{-}III_B$ isolate and its cloned *env* gene products.
Contributor:	Dr. George K. Lewis.
References:	Manuscript in preparation.

Reagent:	**Chessie 6**
Catalog number:	525
Provided:	Amount will be provided on the data sheet sent with the shipment.
Cell type:	Mouse splenocyte/P3X63 Ag8.653 hybridomas.
Medium for propagation:	RPMI 1640 supplemented with 50 μM β-mercaptoethanol, 2000 U/ml penicillin, 200 μg streptomycin, 90%; fetal bovine serum, 10%.
Freeze medium:	Fetal bovine serum, 90%; DMSO, 10%.
Growth characteristics:	Cells grow well either in tissue culture or as ascites in incomplete Freund's adjuvent primed Balb/c mice.
Special characteristics:	Chessie 6 produces a monoclonal antibody of isotype IgG_1. The antibody reacts with gp160 and is specific for gp120 as determined by ELISA and Western blot. This antibody shows a weaker reaction with gp160 than the antibody produced by Chessie 13. It recognizes epitopes which survive alkylation and reduction. Antibody reactivities have been tested using the $HIV\text{-}III_B$ isolate and its cloned *env* gene products.
Contributor:	Dr. George K. Lewis.
References:	Manuscript in preparation.

Reagent: FA_2

Catalog number: 516

Provided: Amount will be provided on the data sheet sent with the shipment.

Cell type: Mouse splenocyte/P3X63 Ag8.653 hybridoma.

Medium for propagation: RPMI 1640 supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, 90%; inactivated fetal bovine serum, 10%.

Freeze medium: RPMI 1640, 80%; fetal bovine serum, 10%; DMSO, 10%.

Growth characteristics: Cells grow best at 3.0-5.0 x 10^5 cells/ml. Split cells once every other day.

Morphology: Cells are characteristically round and larger than small lymphocytes.

Sterility: Negative for bacteria and mycoplasma.

Special characteristics: The monoclonal antibody produced by FA_2 is of isotype IgG_{2b}. It recognizes a 27 kD homologous SIV_{mac} protein and weakly reacts with a 55 kD protein which may be an intermediate in the post-translational processing of SIV *gag* proteins. The antibody does not react with HIV-1, HIV-2, or cloned and uncloned SIV_{agm} antigens, but does react with SIV_{sm} and cloned and uncloned SIV_{mac} antigens.

Contributor: Dr. Suganto Sutjipto and Dr. Preston Marx.

References: Sutjipto, S., et al. *J. Gen. Virol.*, In press.

Reagent: HD_5

Catalog number: 517

Provided: Amount will be provided on the data sheet sent with the shipment.

Cell type: Mouse splenocyte/P3X63 Ag8.653 hybridoma.

Medium for propagation: RPMI 1640 supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, 90%; inactivated fetal bovine serum, 10%.

Freeze medium: RPMI 1640 , 80%; inactivated fetal bovine serum, 10%; DMSO, 10%.

Growth characteristics: Cells grow best at 3.0-5.0 x 10^5 cells/ml. Split cells once every other day.

Morphology: Cells are characteristically round and larger than small lymphocytes.

Sterility: Negative for bacteria and mycoplasma.

Special characteristics: These cells produce a monoclonal antibody of isotype IgG_{2a}. It reacts with a 17kD protein which may correspond to the p16 *gag* SIV protein and shows no reactivity with the *gag* precursor. The antibody does not react with HIV-1, HIV-2, or cloned and uncloned SIV_{agm} antigens, but does react with cloned and uncloned SIV_{mac}, SIV_{sm}, and SIV_{stm} antigens.

Contributor: Dr. Suganto Sutjipto and Dr. Preston Marx.

References: Sutjipto, S., et al. *J. Gen. Virol.*, In press.

Reagent:	**HE3**
Catalog number:	518
Provided:	Amount will be provided on the data sheet sent with the shipment.
Cell type:	Mouse splenocyte/P3X63 Ag8.653 hybridoma.
Medium for propagation:	RPMI 1640 supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, 90%; inactivated fetal bovine serum, 10%.
Freeze medium:	RPMI 1640, 80%; inactivated fetal bovine serum, 10%; DMSO, 10%.
Growth characteristics:	Cells grow best at 3.0-5.0 x 10^5 cells/ml. Split cells once every other day.
Morphology:	Cells are characteristically round and larger than small lymphocytes.
Sterility:	Negative for bacteria and mycoplasma.
Special characteristics:	These cells produce a monoclonal antibody of isotype IgG_{2a}. This antibody reacts with a 27kD homologous SIV_{mac} protein. It also weakly reacts with a 55kD protein which may be an intermediate in the post-translational processing of SIV*gag* proteins. The antibody does not react with HIV-1, HIV-2, or cloned and uncloned SIV_{agm} antigens, but does react with SIV_{sm} and cloned and uncloned SIV_{mac} antigens.
Contributor:	Dr. Suganto Sutjipto and Dr. Preston Marx.
References:	Sutjipto, S., et al. *J. Gen. Virol.*, In press.

Reagent:	**SIM.2**
Catalog number:	511
Provided:	Amount will be provided on the data sheet sent with the shipment.
Cell type:	CB6F1 spleen/P3X63 Ag8.653.
Medium for propagation:	DMEM supplemented with 2 mM L-glutamine, 10 mM HEPES, 0.2 u/ml insulin, 0.05 mg/ml pyruvate, 0.15 mg/ml oxaloacetate, and 5 x 10^{-5} M β-mercaptoethanol, 80%; fetal bovine serum, 10%; NCTC-135, 10%.
Freeze medium:	Fetal bovine serum, 90%, DMSO, 10%.
Growth characteristics:	Cells grow as a single cell suspension. Split 1:10 every 3 to 4 days.
Sterility:	Negative for bacteria and mycoplasma.
Special characteristics:	The antibody produced by these cells recognizes human CD4, recognizes a different epitope from Leu 3a, and blocks syncytium formation. The antibody is of isotype IgG_{2b}, κ chain, and was raised against Sup-T1 cells. The antibody works in immunoprecipitation assays.
Contributor:	Dr. James E.K. Hildreth.
References:	Manuscript in preparation.

Reagent: SIM.4

Catalog number: 512

Provided: Amount will be provided on the data sheet sent with the shipment.

Cell type: CB6F1 spleen/P3X63 Ag8.653.

Medium for propagation: DMEM supplemented with 2.0 mM L-glutamine, 10 mM HEPES, 0.2 u/ml insulin, 0.05 mg/ml pyruvate, 0.15 mg/ml oxaloacetate, and 5×10^{-5} M β-mercaptoethanol, 80%; fetal bovine serum, 10%; NCTC-135, 10%.

Freeze medium: Fetal bovine serum, 90%; DMSO, 10%.

Growth characteristics: Cells grow as a single cell suspension. Split 1:10 every 3 to 4 days.

Sterility: Negative for bacteria and mycoplasma.

Special characteristics: The antibody produced by the cells recognizes human CD4, binds to the same epitope as Leu 3a, and blocks HIV-induced syncytium formation. The antibody is of isotype IgG_1, κ chain, and was raised against Sup-T1 cells. The antibody works in immunoprecipitation assays.

Contributor: Dr. James E.K. Hildreth.

References: Manuscript in preparation.

Reagent: T2C5

Catalog number: 278

Cell type: EBV transformed tonsillar B cells.

Medium for propagation: RPMI 1640, 80%; fetal bovine serum, 20%.

Freeze medium: Fetal bovine serum, 90%, DMSO, 10%.

Reverse transcriptase: Positive.

Special characteristics: This transformed human B cell line was cloned by two consecutive limiting dilutions and secretes monoclonal antibody specific for the *gag* gene product of 55 kD and a protein of 40 kD.

Contributor: Dr. Jay Levy.

References: Evans, L.A., et. al. *J. Immunol.* 140:941, 1988.

CELL LINES, VIRUS INFECTED

Human Immunodeficiency Virus 1

Reagent: ☣ 8E5/LAV

Catalog number: 95

Provided: 2 x 10^6 cells/vial.

Cell type: Subclone of A3.01, a $CD4^+$ CEM derived human T cell line.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: RPMI 1640, 82.5%; fetal bovine serum, 10%; DMSO, 7.5%.

Growth characteristics: When thawing, dilute cells with 37°C medium very slowly in a dropwise fashion. Begin the culture at a high cell density, as cells do not initiate growth well unless they are crowded. Maintain cells at about 10^6 cells/ml.

Morphology: Cells resemble other T cell lines.

Sterility: Negative for bacteria, mycoplasma, and fungi.

Special characteristics: A3.01 cells were infected with LAV and selected by a series of 3 exposures to IUdR. Each contains a single integrated copy of proviral DNA directing synthesis of defective virus particles. No unintegrated DNA.

Contributor: Dr. Thomas Folks.

References: Folks, T.M., et al. *J. Exp. Med.* 164:280, 1986.

Reagent: ☣ **U1/HIV-1**

Catalog number: 165

Provided: 2 x 10^6 cells/vial.

Cell type: Subclone of U937 post-infected promonocyte.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: Propagation medium, 92.5%; DMSO, 7.5%.

Growth characteristics: Cells grow in suspension. Slow growth, one division per 36 hours.

Morphology: Large, semigranular.

Sterility: Negative for bacteria, mycoplasma, and fungi.

Special characteristics: U1 is a subclone of U937 chronically infected with HIV-1 and shows minimal constitutive expression of virus. Certain cytokines and phorbol myristate acetate can induce virus expression. U1 cells can take up and secrete virus into the medium. Surface expression of CD4 is low in cells. Useful for latency induction experiments. Cells should remain in log phase expanded growth (98% viability) immediately prior to stimulation. Supernatant reverse transcriptase activity and viral antigens can be detected approximately 24-48 hours after stimulation.

Contributor: Dr. Thomas Folks.

References: Folks, T.M., et al. *Science* 238:800, 1987.

Reagent: ☣ **ACH-2**

Catalog number: 349

Provided: 2 x 10^6 cells/vial.

Cell type: CEM derivative (A3.01).

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: RPMI 1640, 82.5%; fetal bovine serum, 10%; DMSO, 7.5%.

Growth characteristics: Doubling time is 24 hours.

Sterility: Negative for bacteria, mycoplasma, and fungi.

Special characteristics: HIV-1 latent T cell clone, $CD4^-$, $Leu1^+$, $HIV\text{-}1^+$. Can be induced with PMA, or TNF-α to secrete high levels of infectious HIV-1.

Contributor: Dr. Thomas Folks.

References: Clouse, K.A., et al. *J. Immunol.* 142:431, 1989.
Folks, T.M., et al. *Proc. Natl. Acad. Sci. (USA)* 86:2365, 1989.

Reagent: ☣ **H9/HTLV-III$_B$ NIH 1983**

Catalog number: 400

Provided: 5 x 10^6 cells/vial.

Cell type: Single cell clone derived from HUT 78.

Medium for propagation: RPMI 1640 with L-glutamine, 80%; fetal bovine serum, 20%.

Freeze medium: RPMI 1640 with L-glutamine, 50%; fetal bovine serum, 40%; DMSO, 10%.

Viability: 75%.

Sterility: The cloned cell population was extensively characterized to exclude the presence of adventitious virus and mycoplasma and has been consistently negative in culture since 1984.

Special characteristics: Virus has high capacity for replication in T cell lines. This virus appears to be well adapted for *in vitro* culture in T cell lines and replicates less in fresh human macrophages.

Contributor: Dr. Robert Gallo.

References:
Popovic, M., et al. *Science* **224**:497, 1984.
Popovic, M., Read-Connole, E., and Gallo, R. *Lancet* **ii**:1472, 1984.
Ratner, L., et al. *Nature* **313**:277, 1985.

Reagent: ☣ **H9/HTLV-III$_{RF}$ NIH 1983**

Catalog number: 401

Provided: 5 x 10^6 cells/vial.

Cell type: Single cell clone derived from HUT 78.

Medium for propagation: RPMI 1640 with L-glutamine, 80%; fetal bovine serum, 20%.

Freeze medium: RPMI 1640 with L-glutamine, 50%; fetal bovine serum, 40%; DMSO, 10%.

Sterility: The cloned cell population was extensively characterized to exclude the presence of adventitious virus and mycoplasma and has been consistently negative in culture since 1984.

Special characteristics: This virus strain was obtained from peripheral blood lymphocytes in October 1983. It has been in continuous production in H9 cells since 1984. This strain is more cytopathic than HTLV-III$_B$. There is approximately 10% difference in the nucleic acid sequence from HTLV-III$_B$.

Contributor: Dr. Robert Gallo.

References:
Popovic, M., et al. *Science* **224**:497, 1984.
Starcich, B.R., et al. *Cell* **45**:637, 1986.

Reagent: ☣ **H9/HTLV-III$_{MN}$ NIH 1984**

Catalog number: 402

Provided: 5 x 10^6 cells/vial.

Cell type: Single cell clone derived from HUT 78.

Medium for propagation: RPMI 1640 with L-glutamine, 80%; fetal bovine serum, 20%.

Freeze medium: RPMI 1640 with L-glutamine, 50%; fetal bovine serum, 40%; DMSO, 10%.

Viability: 75%.

Sterility: The cloned cell population was extensively characterized to exclude the presence of adventitious virus and mycoplasma and has been consistently negative in culture since 1984.

Special characteristics: Virus transmitted into H9 cells in March 1984.

Contributor: Dr. Robert Gallo.

References: Gallo, R.C., et al. *Science* **224**:500, 1984.
Shaw, G.M., et al. *Science* **226**:1165, 1984.

Reagent: ☣ **H9/HTLV-III$_{CC}$ NIH 1983**

Catalog number: 403

Provided: 5 x 10^6 cells/vial.

Cell type: Single cell clone derived from HUT 78.

Medium for propagation: RPMI 1640 with L-glutamine, 80%; fetal bovine serum, 20%.

Freeze medium: RPMI 1640 with L-glutamine, 50%; fetal bovine serum, 40%; DMSO, 10%.

Viability: 75%.

Sterility: The cloned cell population was extensively characterized to exclude the presence of adventitious virus and mycoplasma and has been consistently negative in culture since 1984.

Special characteristics: The primary culture of peripheral blood from a patient with dual infection with HTLV-I and HIV-1 was made in February 1983. In 1986 HIV-1 was transmitted to the H9 cell line independent of HTLV-I and further characterized as an early unique isolate.

Contributor: Dr. Robert Gallo.

References: Gallo, R.C., et al. *Nature* **321**:119, 1986.

Reagent: ☣ **HUT 78/HIV-1$_{SF2}$ (a.k.a. ARV-2)**

Catalog number: 279

Provided: 2 x 10^6 cells/vial.

Cell type: Human T cell lymphoma.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: RPMI 1640, 80%; fetal bovine serum, 10%; DMSO, 10%.

Growth characteristics: Cells should be passaged at 1:3 to 1:4 about every 3 days.

Sterility: Negative for bacteria, mycoplasma, and fungi.

Special characteristics: The virus in the cells was isolated from peripheral blood mononuclear cells of an AIDS patient. It infected three human T cell lines and the U937 promonocyte line in a study of HIV cellular tropism and susceptibility to serum neutralization.

Contributor: Dr. Jay Levy.

References: Levy, J.A., et al. *Science* **225**:840, 1984.
Sanchez-Pescador, R., et al. *Science* **227**:484, 1985.

Reagent: ☣ **CEM/AZT-resistant HIV**

Catalog number: 408

Provided: 8 x 10^6 cells/vial.

Cell type: Human T lymphoblastoid cell line.

Medium for propagation: MEM for suspension cultures, 90%; fetal bovine serum, 10%; antibiotic free.

Freeze medium: Propagation medium, 95%; DMSO, 5%.

Growth characteristics: Cells are grown in suspension. An inoculum of 10^5 cells/ml will increase 4-5 fold in 4-5 days when incubated at 37°C, provided pH is maintained at 7.0 and fresh medium is added every other day. Maintenance of cell population at 2-3 x 10^6 cells/ml is optimal for growth. A complete set of instructions for propagation of the infected cells will be included with each shipment.

Special characteristics: Virus was originally isolated from patients with AIDS or ARC who had been treated for a prolonged period with zidovudine. Peripheral blood lymphocytes from patients were co-cultivated with MT-2 cells, and drug sensitivity was examined using HeLa CD4$^+$ cells.

Contributor: Dr. Douglas Richman.

References: Larder, B.A., Darby, G., and Richman, D.D. *Science* 243:1731, 1989.

NOTE: *Aliquots of cells infected with four separate strains will be shipped with a single order.*

Reagent: ☣ **CR10/N1T**

Catalog number: 392

Provided: 5×10^6 cells/vial.

Cell type: T-lymphoid. CR10 cells chronically infected with HIV-1/N1T virus.

Medium for propagation: RPMI 1640, 95%; fetal bovine serum, 5%.

Freeze medium: RPMI 1640, 80%; fetal bovine serum, 10%; DMSO, 10%.

Growth characteristics: Grows as a single cell suspension; doubling time about 24 hours; should be maintained in the range of $0.2\text{-}1.5 \times 10^6$ cells/ml.

Morphology: Round cells; occasional giant cells.

Special characteristics: Chronic producer of a HIV-1/N1T strain of HIV-1. Production level: $1.0\text{-}5.0 \times 10^3$ pg HIV-1 p24/ml, 24 hours after splitting 1:5. Suitable for large-scale production of N1T virus. Does not require addition of uninfected CR10 cells to maintain.

Contributor: Dr. D.J. Volsky.

References: Casareale, D., et al. *AIDS Res.* 1:407, 1985.
Casareale, D., et al. *Virol.* 156:40, 1987.

Human Immunodeficiency Virus 2

Reagent: ☣ **CEMx174/HIV-2ST**

Catalog number: 234

Provided: Amount will be provided on the data sheet sent with the shipment.

Cell type: Somatic cell hybrid culture between CEM and B cell line 174, both of human origin.

Medium for propagation: RPMI 1640, 85%; fetal bovine serum, 15%; pen-strep.

Freeze medium: Propagation medium, 90%; DMSO, 10%.

Growth characteristics: Cells grow in large firm clumps which are difficult to dissociate.

Morphology: Larger and more oblong than CEM parent.

Sterility: Negative for bacteria, mycoplasma, and fungi.

Special characteristics: These cells express B cell, T cell, and class II HLA markers. They were infected by phage clone pJSP4-27 of HIV-2ST.

Contributor: Dr. Beatrice Hahn and Dr. George Shaw.

References: Kong, L.I., et al. *Science* 240:1525, 1988.

Reagent: ☣ U937/HIV-2$_{MS}$

Catalog number: 127

Provided: 7×10^6 cells/vial.

Cell type: Human histiocytic lymphoma.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: Propagation medium, 90%; DMSO, 10%.

Growth characteristics: Seeding ratio is 1:10.

Morphology: Monocyte-like.

Special characteristics: Cells are infected with HIV-2$_{MS}$.

Contributor: Dr. Phyllis Kanki.

References: Kanki, P., Barrin, F., and Essex, M. Abst. #1659, Fourth International Conference on AIDS, 1988.

HHV-6

Reagent:	☣ HSB-2/HHV-6GS
Catalog number:	350
Provided:	8×10^6 cells/vial.
Cell type:	Human T cell lymphoblast line.
Medium for propagation:	RPMI 1640, 90%; fetal bovine serum, 10%, antibiotic free.
Freeze medium:	Propagation medium, 95%; DMSO, 5%.
Viability:	70%.
Growth characteristics:	Grow infected cells in suspension. Maintain at 5×10^5 cells/ml. When cytopathic effects begin to occur, add uninfected cells at a ratio of 9 to every infected cell. A set of instructions for the proper thawing and propagation of the infected cells will be included with the shipment.
Morphology:	Lymphoblast-like.
Sterility:	Negative for bacteria, mycoplasma, and fungi.
Special characteristics:	HHV-6 was originally isolated from peripheral blood leukocytes under the name human B-lymphotrophic virus (HBLV). The specific strain offered is GS. Although HSB-2 cells are productively infected with HHV-6, cytopathic effects are observed and fresh cells must be continually added to ensure viral propagation. The researcher should be aware that while the GS strain grows in HSB-2 cells, not all strains will. Other strains of HHV-6 should be grown in human cord blood lymphocytes to ensure viral propagation.
Contributor:	Dr. Robert Gallo.
References:	Ablashi, D.V., et al. *Nature* 329:207, 1987. Ablashi, D.V., et al. *Int. J. Cancer* 42:787, 1988. Lusso, P., et al. *Nature* 337:370, 1989. Salahuddin, S.Z., et al. *Science* 234:596, 1986.

NOTE: *The uninfected HSB-2 cells are available as catalog #497.*

Distributed with the permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.

A patent application has been filed on the use of the HSB-2 cell line to produce HHV-6. Corporate requests should be directed to Dr. Joseph Rosebrock at (301)622-4218.

HTLV-I

Reagent: ☣ C8166-45

Catalog number: 404

Provided: 5×10^6 cells/vial.

Cell type: Human umbilical cord blood lymphocytes.

Medium for propagation: RPMI 1640 with L-glutamine, 90%; fetal bovine serum, 10%. No IL-2 is required.

Freeze medium: RPMI 1640 with L-glutamine, 83%; fetal bovine serum, 10%; DMSO, 7%.

Sterility: Negative for bacteria, mycoplasma, and adventitious virus.

Special characteristics: This cell line carries, but does not express, the HTLV-I genome which has been used as a target for HIV-1 infection. It is denoted as C63/CRII-2 in the reference.

Contributor: Dr. Robert Gallo.

References: Salahuddin, S.Z., et al. *Virol.* 129:51, 1983.

Reagent: ☣ MT-2

Catalog number: 237

Provided: 2×10^6 cells/vial.

Cell type: Human T cell leukemia cells.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: RPMI 1640, 70%; fetal bovine serum, 20%; DMSO, 10% .

Growth characteristics: Split twice a week. Cells grow in clumpy suspension.

Reverse transcriptase: Positive.

Special characteristics: Transformed with and continuous producer of HTLV-I. Line cloned for maximal cytopathic effects with LAV-1 and cured of mycoplasma by Dr. John Riggs, Virology Laboratory, California Department of Public Health, Berkeley, California.

Contributor: Dr. Douglas Richman.

References: Harada, S., Koyanagi, Y. and Yamamoto, N. *Science* 229:563, 1985.

Reagent:	☣ **MT-4**
Catalog number:	120
Provided:	2 x 10^6 cells/vial.
Cell type:	Human T cell leukemia cells.
Medium for propagation:	RPMI 1640, 90%; fetal bovine serum, 10%.
Freeze medium:	RPMI 1640, 70%; fetal bovine serum, 20%; DMSO, 10%.
Growth characteristics:	Cells grow in suspension. Passage cells one to two times weekly.
Morphology:	T lymphoblast cells.
Reverse transcriptase:	Negative.
Special characteristics:	HTLV-I transformed. Reverse transcriptase production negative or sufficiently low to assay for production of RT. Very useful for cytotoxicity inhibition assays for antiviral drugs.
Contributor:	Dr. Douglas Richman.
References:	Harada, S., Koyanagi, Y., and Yamamoto, N. *Science* **229**:563, 1985. Larder, B.A., Darby, G., and Richman, D.D. *Science* **243**:1731, 1989. Pauwels, R., et al. *J. Virol. Methods* **16**:171, 1987.

Simian Immunodeficiency Virus

Reagent:	☣ **HUT 78/SIV$_{mac}$251**
Catalog number:	160
Provided:	2 x 10^6 cells/vial.
Cell type:	Human cutaneous T cell lymphoma.
Medium for propagation:	RPMI 1640, 90%; fetal bovine serum, 10%.
Freeze medium:	Propagation medium, 90%; DMSO, 10%.
Growth characteristics:	Cells are passaged twice a week at 1:2 to 1:3.
Morphology:	Similar to parent line.
Sterility:	Negative for bacteria, mycoplasma, and fungi.
Special characteristics:	Uninfected HUT 78 cells are added to the culture every 7-14 days to maintain maximal RT activity in supernatant.
Contributor:	Dr. Ronald Desrosiers.
References:	Daniel, M.D., et al. *Science* **228**:1201, 1985.

Reagent: ☣ **H9/SIV$_{mac}$186**

Catalog number: 161

Provided: 2x10^6 cells/vial.

Cell type: Human T cell.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: Propagation medium, 90%; DMSO, 10%.

Growth characteristics: Cells are passaged twice a week at 1:2 to 1:3.

Morphology: Similar to parent line.

Sterility: Negative for bacteria, mycoplasma, and fungi.

Special characteristics: Uninfected H9 cells are added to the cultures every 7-14 days to maintain RT activity in supernatant.

Contributor: Dr. Ronald Desrosiers.

References:
Daniel, M.D., et al. *Int. J. Cancer* 41:601, 1988.
Kestler III, H.W., et al. *Nature* 331:619, 1988.

Reagent: ☣ **Molt 4 Clone 8/SIV$_{agm}$**

Catalog number: 174

Provided: 2 x 10^6 cells/vial.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%; antibiotics.

Freeze medium: RPMI 1640, 80%; fetal bovine serum, 10%; DMSO, 10%.

Growth characteristics: Split twice a week 1:4 or 1:5.

Sterility: Negative for bacteria, mycoplasma, and fungi.

Contributor: Dr. Ronald Desrosiers.

References:
Daniel, M.D., et al. *J. Virol.* 62:4123, 1988.

Reagent: ☣ **H9/SIV$_{smm}$smH-3**

Catalog number: 460

Provided: 8 x 10^6 cells/vial.

Cell type: H9 (a single-cell clone derived from HUT 78).

Medium for propagation: RPMI 1640 with L-glutamine, 90%; fetal bovine serum (heat inactivated), 10%; pen-strep.

Freeze medium: Fetal bovine serum, 90%; RPMI 1640, 10%.

Growth characteristics: Split 1:4 twice a week. Keep cells at 0.25-1.0 x 10^6cells/ml.

Morphology: Lymphocyte.

Sterility: Negative for bacteria and fungi.

Special characteristics: These cells produce sooty mangabey viruses which were originally used to investigate evolutionary diversity of lentiviruses. They can be used for comparison to other retroviral isolates (HIV-1, HIV-2, or SIV).

Contributor: Dr. Philip Johnson.

References: Hirsh, V.M., et al. *Nature* 339:389, 1989.

Reagent: ☣ **H9/SIV$_{smm}$smH-4**

Catalog number: 461

Provided: 8 x 10^6 cells/vial.

Cell type: H9 (single-cell clone derived from HUT 78).

Medium for propagation: RPMI 1640 with L-glutamine, 90%; fetal bovine serum (heat inactivated), 10%; pen-strep.

Freeze medium: Fetal bovine serum, 90%; RPMI 1640, 10%.

Growth characteristics: Split 1:4 twice a week. Keep cells at 0.25-1.0 x 10^6cells/ml.

Morphology: Lymphocyte.

Sterility: Negative for bacteria and fungi.

Special characteristics: These cells produce sooty mangabey viruses which were originally used to investigate evolutionary diversity of lentiviruses. They can be used for comparison to other retroviral isolates (HIV-1, HIV-2, or SIV).

Contributor: Dr. Philip Johnson.

References: Hirsch, V.M., et al. *Nature* 339:389, 1989.

Reagent: ☣ **HUT 78/SIV-BK28**

Catalog number: 173

Provided: 2 x 10^6 cells/vial.

Cell type: Human cutaneous T cell lymphoma.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze medium: MEM with 50% fetal bovine serum, 90%; DMSO, 10%.

Growth characteristics: Inoculum of 5 x 10^6 cells in a 75 cm^2 flask yields approximately 18-20 x 10^6 cells in a week.

Morphology: See special characteristics.

Sterility: Negative for bacteria, mycoplasma, and fungi.

Special characteristics: When provirus is introduced into cells by transfection, cellular atypia is observed on day 5 and multinucleated giant cells are observed by day 10. RT activity peaks at 10 days and remains elevated for more than 90 days; morphological changes diminish progressively. No significant cytolysis is observed. The plasmid clone pBK28-SIV (catalog #133) is also available.

Contributor: Dr. James I. Mullins.

References: Kornfield, H., et al. *Nature* **326**:610, 1987.

Reagent: ☣ **HUT 78/BK44**

Catalog number: 312

Provided: 2 x 10^6 cells/vial.

Cell type: Human cutaneous T cell lymphoma.

Medium for propagation: RPMI 1640, 90%; fetal bovine serum, 10%; antibiotics.

Freeze medium: Ice cold solution of RPMI 1640, 40%; DMSO, 10%; fetal bovine serum, 50%; antibiotics.

Growth characteristics: Grows in clumps. Maintain the culture at 10^5 to 10^6 cells/ml.

Sterility: Negative for bacteria and mycoplasma.

Special characteristics: To optimally maintain cells, at time of passage feed with a medium consisting of 50% spent medium and 50% fresh medium.

Contributor: Dr. James I. Mullins.

References: Kornfield, H., et al. *Nature* **326**:610, 1987.

Feline Leukemia Virus

Reagent:	☣ **AH927/61E**
Catalog number:	168
Provided:	2 x 10^6 cells/vial.
Cell type:	Feline embryo fibroblasts.
Medium for propagation:	MEM, 90%; fetal bovine serum, 10%.
Freeze medium:	MEM with 50% fetal bovine serum, 90%; DMSO, 10%.
Growth characteristics:	Inoculum of 5 x 10^6 cells in a 75 cm^2 flask yields approximately 12-15 x 10^6 cells in a week.
Morphology:	Epithelial-like.
Sterility:	Negative for bacteria, mycoplasma, and fungi.
Special characteristics:	AH927 cells were obtained from Dr. S. Rasheed, UCLA, and were transfected with cloned FeLV 61E DNA. RT is detected in supernatant by 12 days, as is proviral DNA. The plasmid clone 61E (catalog #109) is also available.
Contributor:	Dr. James I. Mullins.
References:	Overbaugh, J., et al. *Science* **239**:906, 1988.

Reagent:	☣ **AH927/EECC**
Catalog number:	170
Provided:	2 x 10^6 cells/vial.
Cell type:	Feline embryo fibroblasts.
Medium for propagation:	MEM, 90%; fetal bovine serum, 10%.
Freeze medium:	MEM with 50% fetal bovine serum, 90%; DMSO, 10%.
Growth characteristics:	Inoculum of 5 x 10^6 cells in a 75 cm^2 flask yields approximately 12-15 x 10^6 cells in a week.
Morphology:	Epithelial-like.
Sterility:	Negative for bacteria, mycoplasma, and fungi.
Special characteristics:	AH927 cells were obtained from Dr. S. Rasheed, UCLA, and transfected with cloned FeLV EECC DNA. RT is detected in supernatant by 12 days, as is proviral DNA. The plasmid clone pEECC (catalog #105) is also available.
Contributor:	Dr. James I. Mullins.
References:	Overbaugh, J., et al. *Science* **239**:906, 1988.

Reagent: ☣ **3201/EECC**
Catalog number: 197
Provided: 2 x 10^6 cells/vial.
Cell type: Feline T cell.
Medium for propagation: RPMI 1640, 45%; Leibovitz's L-15, 45%; fetal bovine serum, 10%.
Freeze medium: Propagation medium with 50% fetal bovine serum, 90%; DMSO, 10%.
Growth characteristics: Grows in clumps.
Morphology: T cell-like.
Sterility: Negative for bacteria, mycoplasma, fungi, and yeast.
Contributor: Dr. James I. Mullins.
References: Snyder, Jr., H.W., et al. *Nature* 275:656, 1978.

Reagent: ☣ **3201/61E**
Catalog number: 198
Provided: 2 x 10^6 cells/vial.
Cell type: Feline T cells.
Medium for propagation: RPMI 1640, 45%; Leibovitz's L-15, 45%; fetal bovine serum, 10%.
Freeze medium: Propagation medium with 50% fetal bovine serum, 90%; DMSO, 10%.
Growth characteristics: Grows in clumps.
Morphology: T cell-like.
Sterility: Negative for bacteria, mycoplasma, and fungi.
Contributor: Dr. James I. Mullins.
References: Snyder, Jr., H.W., et al. *Nature* 275:656, 1978.

VIRUS ISOLATES

Human Immunodeficiency Virus 1

Reagent: ☣ HIV-1_{BR}

Catalog number: 390

Provided: 1 vial cell-free virus.

Strain: BR

Original source: Autopsied brain tissue of a patient who suffered from progressive dementia.

Preparation: Cleared culture supernatant from infected human PBMC (peripheral blood mononuclear cells). PBMC were stimulated with PHA for 2 days, virus infected, and cultivated in the presence of IL-2.

Host of choice: Human PBMC.

Host range: Human PBMC, monocytoid cell lines ROHA and U937.

Special characteristics: Cytopathic to T4 positive cells. Replication competent. Has a 13 amino acid-encoding sequence duplicated in the *nef* gene.

Contributor: Dr. Rita Anand.

References: Anand, R., et al. *Virol.* **168**:79, 1989.

NOTE: ***NOTE: The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.***

Reagent: ☣ HIV-1_{JR-CSF}

Catalog number: 394

Provided: 1 vial cell-free virus.

Original source: Filtered cerebrospinal fluid of patient with AIDS dementia.

Preparation: Infection of PHA-stimulated primary human peripheral blood lymphocytes (PBL), harvested 1 week following infection from a 24-hour culture supernatant.

Host of choice: Primary human PBL.

Host range: Primary human PBL and mononuclear phagocytes (less efficient than HIV-1_{JR-FL}).

Special characteristics: Molecularly cloned following short-term passage in PBL. Virus is derived from transfection of molecular clone into 729 B-cells and rescued by cocultivation with primary human PBL. HIV-1_{JR-CSF} will not productively infect any cell lines tested.

Contributor: Dr. Irvin S.Y. Chen.

References: Koyanagi, Y., et al. *Science* **236**:819, 1987.

Reagent: ☣ **HIV-1$_{JR-FL}$**

Catalog number: 395

Provided: 1 vial cell-free virus.

Original source: Frontal lobe brain tissue of patient with AIDS dementia obtained at autopsy.

Preparation: Infection of PHA-stimulated primary human peripheral blood lymphocytes (PBL), harvested one week following infection from a 24 hour culture supernatant.

Host of choice: Primary human PBL.

Host range: Primary human PBL and primary human mononuclear phagocytes.

Special characteristics: Will not replicate in any cell lines tested, including Jurkat, HUT 78 and U937.

Contributor: Dr. Irvin S.Y. Chen.

References: Koyanagi, Y., et al. *Science* **236**:819, 1987.

Reagent: ☣ **HTLV-III$_{RF}$/H9**

Catalog number: 316

Provided: 1 vial cell-free virus.

Strain: RF

Original source: Peripheral blood lymphocytes.

Preparation: Tissue culture supernatants from infected H9 cells.

Host of choice: H9.

Host range: Human neoplastic $CD4^+$ T cells including H9, CEM, U937, Molt 3, HeLa $CD4^+$ cells, and peripheral blood lymphocytes.

Contributor: Dr. Robert Gallo.

References: Popovic, M., et al. *Science* **224**:497, 1984.
Starcich, B., et al. *Cell* **45**:637, 1986.

Reagent: ☣ **HTLV-III$_{MN}$/H9**

Catalog number: 317

Provided: 1 vial cell-free virus.

Strain: MN

Original source: Peripheral blood lymphocytes.

Preparation: Tissue culture supernatant from infected H9 cells.

Host of choice: H9.

Host range: Human neoplastic $CD4^+$ T cells including H9, CEM, U937, Molt 3, HeLa $CD4^+$ cells, and peripheral blood lymphocytes.

Contributor: Dr. Robert Gallo.

References:
Gallo, R.C., et al. *Science* **224**:500, 1984.
Shaw, G.M., et al. *Science* **226**:1165, 1984.

Reagent: ☣ **HTLV-III$_{B}$/H9**

Catalog number: 398

Provided: 1 vial cell-free virus.

Strain: B

Original source: Peripheral blood or bone marrow from patients with AIDS or related diseases.

Preparation: Concentrated culture fluids of peripheral blood or bone marrow from several patients with AIDS or related diseases were used to establish a permanent productive infection in a cloned permissive neoplastic T cell line (H9).

Host of choice: H9.

Host range: Human neoplastic $CD4^+$ T cells including H9, CEM, U937, Molt 3, HeLa $CD4^+$ cells, and peripheral blood lymphocytes.

Special characteristics: High capacity to replicate in T cell lines. This virus appears to be well adapted for *in vitro* culture in T cell lines and replicates less in fresh human macrophages.

Contributor: Dr. Robert Gallo.

References:
Popovic, M., et al. *Science* **224**:497, 1984.
Popovic, M., Read-Connole, E., and Gallo, R.C. *Lancet* **ii**:1472, 1984.
Ratner, L., et al. *Nature* **313**:277, 1985.

Reagent: ☣ **HTLV-III$_{CC}$/H9**

Catalog number: 399

Provided: 1 vial cell-free virus.

Strain: CC

Original source: Peripheral blood from an AIDS patient.

Preparation: The primary culture of peripheral blood from a patient with dual infection with HTLV-I and HIV-1 was made in February, 1983. In 1986 HIV-1 was transmitted to the H9 cell line independent of HTLV-I and further characterized as an early unique isolate.

Host of choice: H9.

Host range: Human neoplastic $CD4^+$ T cells including H9, CEM, U937, Molt 3, HeLa $CD4^+$ cells, and peripheral blood lymphocytes.

Contributor: Dr. Robert Gallo.

References: Gallo, R.C., et. al. *Nature* **321**:119, 1986.

Reagent: ☣ **HIV-1$_{Ba-L}$**

Catalog number: 510

Provided: 1 vial cell-free virus.

Strain: Ba-L

Original source: Human infant lung tissue.

Preparation: Primary culture of plastic-adherent, nonspecific esterase positive cells.

Host of choice: Human peripheral blood-derived monocytes/macrophages.

Host range: Human peripheral blood-derived monocytes/macrophages, peripheral blood $CD4^+$ lymphocytes.

Special characteristics: Ba-L can be propagated to high titers only in normal human peripheral blood-derived monocyte/macrophage. Maintain monocytes/macrophages in RPMI 1640 supplemented with 0.16 μM L-glutamine, 20% heat inactivated fetal bovine serum, and 50 U/ml pen-strep.

Contributor: Dr. Suzanne Gartner, Dr. Mikulas Popovic, and Dr. Robert Gallo.

References: Gartner, S., et al. *Science* **233**:215, 1986.
Popovic, M., et al. *Retroviruses of Human AIDS and Related Animal Diseases; Colloque Des Cent Gardes, October 27-29, 1988*. Edited by Girard, M. and Valette, L., 21-27. Paris: Marnes-La-Coquette, 1989.

Reagent: ☣ **HIV-1$_{24\ MO}$ Monocytropic Virus**

Catalog number: 416

Provided: 1 vial cell-free virus.

Strain: 24 MO

Original source: Seropositive AIDS patient.

Preparation: Confluent blood-derived monocyte/macrophage monolayers were treated with recombinant M-CSF and incubated with supernatant fluid from viral cultures. Virus was harvested daily and p24 antigen levels were maintained.

Host of choice: Human monocytes/macrophages.

Host range: Will grow in peripheral blood lymphocytes.

Special characteristics: Titer is 10^2 - 10^3 $TCID_{50}$/ml. Preparation is negative for bacteria and mycoplasma.

Contributor: Dr. Howard Gendelman.

References: Gendelman, H.E., et al. *AIDS* **3**:475, 1989.
Gendelman, H.E., et al. *J. Exp. Med.* **167**:1428, 1988.

Reagent: ☣ **HIV-1$_{SF2}$ (a.k.a. ARV-2)**

Catalog number: 275

Provided: 1 vial cell-free virus.

Strain: SF2

Original source: Peripheral blood mononuclear cells (PBMC) from patient with AIDS.

Preparation: Patient's PBMC were co-cultured with mitogen-stimulated PBMC from seronegative donors.

Host of choice: Human cells.

Special characteristics: This isolate from PBMC could infect all three human T cell lines used in the study (see reference) as well as U937. Serum neutralization pattern was similar to other PBMC isolates.

Contributor: Dr. Jay Levy.

References: Cheng-Mayer, C. and Levy, J.A. *Ann. Neurol.* **23**:S58, 1988.

Reagent: ☣ **HIV-1 SF_{162}**

Catalog number: 276

Provided: 1 vial cell-free virus.

Strain: SF_{162}

Original source: Cerebrospinal fluid of patient with AIDS.

Preparation: Patient's CSF was co-cultured with mitogen-stimulated PBMC (peripheral blood mononuclear cells) from seronegative donors.

Host of choice: Human cells.

Special characteristics: This isolate from CSF did not infect human T cell lines or U937, and was not easily neutralized by HIV antibody-positive sera. It grows in peripheral blood macrophages.

Contributor: Dr. Jay Levy.

References: Cheng-Mayer, C. and Levy, J.A. *Ann. Neurol.* **23**:S58, 1988.

Reagent: ☣ **HIV-1 (NL4-3/A3.01)**

Catalog number: 78

Provided: 1 vial cell-free virus.

Strain: Chimeric NY5' and LAV 3' fused at the *Eco*RI site.

Original source: Total genomic DNA from NY5 and LAV.

Preparation: SW480 and A3.01 lines were transfected with molecularly cloned DNA. Forty eight hours post-transfection virus particles were harvested and passaged into A3.01 cells. At peak reverse transcriptase supernatant was harvested and frozen at -70°C.

Host of choice: SW480 and A3.01.

Host range: Mouse, mink, monkey, and several non-T cell lines.

Special characteristics: Upon infection this virus directed the production of infectious virus particles in a wide variety of cells. The progeny, infectious virions, were synthesized in mouse, mink, monkey, and several non-T cell lines, indicating the absence of any intracellular obstacle to viral RNA or protein production or assembly.

Contributor: Dr. Malcolm Martin.

References: Adachi, A., et al. *J. Virol.* **59**:284, 1986.

Reagent: ☣ **LAV.04/A3.01**
Catalog number: 235
Provided: 1 vial cell-free virus.
Strain: LAV-1
Original source: Patient with AIDS; sent to Dr. Martin by Dr. L. Montagnier, France.
Preparation: Filtered, infectious culture fluid from infected A3.01 cells.
Host of choice: Human peripheral blood mononuclear cells or $CD4^+$ T cell lines.
Special characteristics: This virus is highly cytopathic.
Contributor: Dr. Malcolm Martin.
References: Barre-Sinoussi, F., et al. *Science* **220**:868, 1983.

Human Immunodeficiency Virus 2

Reagent: ☣ **HIV-2_{MS}/U937**
Catalog number: 98
Provided: 1 vial cell-free virus.
Strain: MS
Original source: Material from patient MS.
Preparation: Supernatant from HIV-2_{MS} infected U937 cells was filtered (0.45 μm pores) and frozen in aliquots at -90°C.
Host of choice: U937.
Host range: Virus can be grown in Sup-T1, Jurkat or any T cell line.
Special characteristics: Growth of HIV-2_{MS} in U937 allows preparation of high titer virus stocks.
Contributor: Dr. Phyllis Kanki.
References: Kanki, P., et al. Abst. #1659, Fourth International Conference on AIDS, 1988.

Simian Immunodeficiency Virus

Reagent: ☣ **$SIV_{mac}251/HUT\ 78$**

Catalog number: 253

Provided: 1 vial cell-free virus.

Strain: *Macaca mulatta*

Original source: Splenic lymphocytes from *Macaca* co-cultured with HUT 78 cells.

Host of choice: Human PBL, H9, HUT 78, CEMx174, Molt 4 Clone 8.

Contributor: Dr. Ronald Desrosiers.

References: Daniel, M.D., et al. *Science* **228**:1201, 1985.

Feline Immunodeficiency Virus

Reagent: ☣ **FIV/FPBM**

Catalog number: 236

Provided: 1 vial cell-free virus.

Strain: Petaluma

Original source: Specific pathogen-free cat (#2429) inoculated with plasma from Petaluma stray cat cattery.

Preparation: FIV was grown in Con A-stimulated feline peripheral blood mononuclear cells maintained on IL-2 and supernatant fluid was harvested and sterile filtered.

Host of choice: Feline peripheral blood lymphocytes, Crandell Feline Kidney Cells (CRFK).

Host range: Feline peripheral blood lymphocytes, CRFK.

Special characteristics: May express feline syncytium-forming virus and feline infectious peritonitis virus upon prolonged culture conditions.

Contributor: Dr. Niels Pedersen and Dr. Janet Yamamoto.

References: Pedersen, N.C., et al. *Science* **235**:790, 1987.

Vaccinia Virus

Reagent: ☣ **Vaccinia**

Catalog number: 353

Provided: 1 vial cell-free virus.

Strain: WR

Original source: ATCC.

Host of choice: HeLa and other vertebrate cells.

Host range: Wide. Human and other vertebrates.

Special characteristics: Mouse neurotropic strain.

Contributor: Dr. Bernard Moss.

References: Parker, R.F., Bronson, L.H., and Green, R.H. *J. Exp. Med.* **74**:263, 1941.

NOTE: ***This strain of vaccinia serves as a control for catalog numbers 354-362.***

GENETIC CLONES

Lambda Clones

Human Immunodeficiency Virus 1

Reagent: **WMF I-16**

Catalog number: 27

Provided: 2 ml of transformed bacteria.

Cloning vector: λgtWesλB.

Bacterial host: LE 392 or equivalent.

Cloning site: *Sac*I.

Titer of growing stock: 1×10^9 pfu/ml.

Source of provirus: λ phage library prepared from total genomic DNA of infected cell culture derived from a heterosexual woman (WMF) with ARC.

Description of clone: Non-permuted, full length provirus cleaved with *Sac*I in the LTR (R) region; transfection competent in appropriate construct.

Special characteristics: Proviral DNA is infectious upon transfection when subcloned in pHBX2D or equivalent.

Contributor: Dr. Beatrice Hahn and Dr. George M. Shaw.

References: Saag, M.S., et al. *Nature* **334**:440, 1988.

NOTE: ***Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **WMF III-3**

Catalog number: 28

Provided: 2 ml of transformed bacteria.

Cloning vector: λgtWesλB.

Bacterial host: LE 392 or equivalent.

Cloning site: *Sac*I.

Titer of growing stock: 1×10^9 pfu/ml.

Source of provirus: λ phage library prepared from total genomic DNA of infected cell culture derived from a heterosexual woman (WMF) with ARC.

Description of clone: Non-permuted, full length provirus cleaved with *Sac*I in LTR (R) region; transfection competent in the appropriate construct.

Special characteristics: In present construct, proviral DNA is infectious upon transfection when subcloned in pHBX2gpt or equivalent.

Contributor: Dr. Beatrice Hahn and Dr. George M. Shaw.

References: Saag, M.S., et al. *Nature* **334**:440, 1988.

NOTE: ***Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **λBH10**

Catalog number: 66

Provided: 2 ml of transformed bacteria.

Cloning vector: λgtWesλB.

Bacterial host: LE 392 or equivalent.

Cloning site: *Sac*I.

Titer of growing stock: 5×10^8 pfu/ml.

Source of provirus: λ phage library prepared from total genomic DNA of infected cell culture designated H9/HTLV-III_B (Popovic et al. *Science* **219**:856, 1983).

Description of clone: Provirus cleaved with *Sac*I in the untranslated leader sequence (5' end) and the LTR (R) region (3' end) is approximately 190 bp shorter than complete genome.

Special characteristics: Not infectious in present form; proviral DNA is infectious upon transfection when subcloned in pHXB2Dgpt or equivalent.

Contributor: Dr. Beatrice Hahn and Dr. George M. Shaw.

References: Hahn, B.H., et al. *Nature* **312**:166, 1984.

NOTE: ***Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **WMJ III-3**

Catalog number: 67

Provided: 2 ml of transformed bacteria.

Cloning vector: λgtWesλB.

Bacterial host: LE 392 or equivalent.

Cloning site: *Sac*I.

Titer of growing stock: 1×10^9 pfu/ml.

Source of provirus: λ phage library prepared from total genomic DNA of infected cell culture derived from an infant (WMJ) with AIDS.

Description of clone: Provirus cleaved with *Sac*I in the untranslated leader sequence (5' end) and the LTR (R) region (3' end).

Special characteristics: Not infectious in present form; proviral DNA is infectious when subcloned in pHBX2Dgpt or equivalent.

Contributor: Dr. Beatrice Hahn and Dr. George M. Shaw.

References: Hahn, B.H., et al. *Science* **232**:1548, 1986.

NOTE: ***Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **λHXB2**

Catalog number: 70

Provided: 2 ml of transformed bacteria.

Cloning vector: J1 lambda.

Bacterial host: LE 392 or equivalent.

Cloning site: *Xba*I.

Titer of growing stock: 4×10^8 pfu/ml.

Source of provirus: λ phage library prepared from total genomic DNA of infected cell culture H9/HTLV-III_B (Popovic et al. *Science* **219**:856, 1983).

Description of clone: Complete provirus with flanking cellular sequences.

Special characteristics: Infectious upon transfection into appropriate cell line.

Contributor: Dr. Beatrice Hahn and Dr. George M. Shaw.

References: Shaw, G.M., et al. *Science* **226**:1165, 1984.

NOTE: ***Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent:	**λHXB3**
Catalog number:	72
Provided:	2 ml of transformed bacteria.
Cloning vector:	J1 lambda.
Bacterial host:	LE 392 or equivalent.
Cloning site:	*Xba*I.
Titer of growing stock:	4×10^8 pfu/ml.
Source of provirus:	Total genomic DNA of infected cell culture H9/HTLV-III$_B$ (Popovic et al. *Science* **219**:856,1983).
Description of clone:	Complete provirus with flanking cellular sequences.
Special characteristics:	Infectious upon transfection into appropriate cell line.
Contributor:	Dr. Beatrice Hahn and Dr. George M. Shaw.
References:	Shaw, G.M., et al. *Science* **226**:1165, 1984.

NOTE: *Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.*

Reagent:	**λRJS-IV.16**
Catalog number:	76
Provided:	2 ml of transformed bacteria.
Cloning vector:	λgtWesλB.
Bacterial host:	LE 392 or equivalent.
Cloning site:	*Eco*RI.
Titer of growing stock:	1.5×10^9 pfu/ml.
Source of provirus:	Infected cellular DNA derived from a primary PBL culture of a homosexual man with ARC.
Contributor:	Dr. Beatrice Hahn and Dr. George M. Shaw.
References:	Saag, M.S., et al. *Nature* **334**:440, 1988.

NOTE: *Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.*

Reagent: **HAT-3**

Catalog number: 81

Provided: 2 ml of transformed bacteria.

Cloning vector: λgtWesλB.

Bacterial host: LE 392 or equivalent.

Cloning site: *Sac*I.

Titer of growing stock: 3×10^8 pfu/ml.

Source of provirus: λ phage library prepared from total genomic DNA of infected cell culture designated H9/HTLV-III_{RF} (Popovic et al., *Science* **219**:856, 1983).

Description of clone: HAT-3 does not contain the *Sac*I site in the 5' leader sequence and therefore represents a full length clone with one LTR. HAT-3 also contains a mutation in the *gag* open reading frame.

Special characteristics: Not infectious because of mutation in *gag* open reading frame.

Contributor: Dr. Beatrice Hahn and Dr. George M. Shaw.

References: Hahn, B.H., et al. *Proc. Natl. Acad. Sci. (USA)* **82**:4813, 1985.

NOTE: ***Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Plasmid Clones

Human Immunodeficiency Virus 1

Reagent: **pBH10 (plasmid clone)**

Catalog number: 90

Provided: 1 vial of transformed bacteria.

Cloning vector: SP64.

Bacterial host: DH-1.

Cloning site: *Sac*I.

Source of provirus: Infected cell line H9/HTLV-III_B.

Description of clone: The viral insert of λBH-10 was excised and subcloned into the *Sac*I site of SP64 (commercially available through Promega Biotech). Contains amp^r marker.

Special characteristics: Not infectious in present form; proviral DNA is infectious upon transfection when subcloned in pHXB2Dgpt or equivalent.

Contributor: Dr. Beatrice Hahn and Dr. George M. Shaw.

References: Hahn, B.H., et al. *Nature* **312**:166, 1984.

NOTE: ***Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent:	**pWMJ II-12 *Sal*I 5'/ *Sst*I 3'**
Catalog number:	131
Provided:	1 vial of transformed bacteria.
Cloning vector:	SP64.
Bacterial host:	HB101.
Cloning site:	*Sal*I (5') and *Sst*I (3') in polylinker SP64.
Source of provirus:	Infected cell line H9/WMJ II.
Description of clone:	The viral insert of λWMJ II-12 was excised with *Sac*I, cleaved with *Sal*I and the 3' fragment (3.5 kb) subcloned into SP64. Contains amp^r marker.
Special characteristics:	Not infectious in present vector. DNA is infectious when subcloned in pHXB2Dgpt or equivalent.
Contributor:	Dr. Beatrice Hahn and Dr. George M. Shaw.
References:	Hahn, B.H., et al. *Science* **232**:1548, 1986.

NOTE: ***Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent:	**pWMJ II-12 *Sst*I 5'/*Sal*I 3'**
Catalog number:	132
Provided:	1 vial of transformed bacteria.
Cloning vector:	SP64.
Bacterial host:	HB101.
Cloning site:	*Sst*I (5') and *Sal*I (3') in polylinker of SP64.
Source of provirus:	Infected cell line H9/WMJ II.
Description of clone:	The viral insert of λWMJ II-12 was excised with *Sac*I, cleaved with *Sal*I and the 5' fragment (5.5 kb) subcloned into SP64. Contains amp^r marker.
Special characteristics:	Not infectious in present vector.
Contributor:	Dr. Beatrice Hahn and Dr. George M. Shaw.
References:	Hahn, B.H., et al. *Science* **232**:1548, 1986.

NOTE: ***Distributed with permission of Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **pPolo**

Catalog number: 238

Provided: 1 vial of transformed bacteria.

Cloning vector: pBR322, derivative pExc13.

Bacterial host: HB101 (K12).

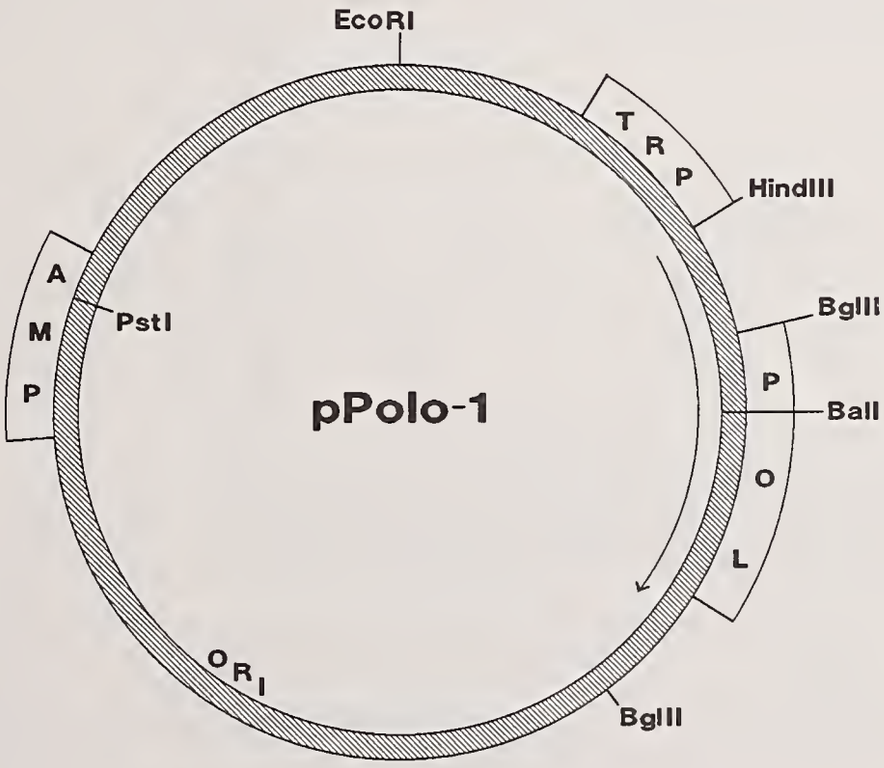
Cloning site: *Bgl*II.

Description of clone: Contains a *Bgl*II-*Bgl*II insert of 4978 bp from HIV-1, seq. 2093-7071. *Pol* ORF extends from 2093-5126. Contains amp^r marker.

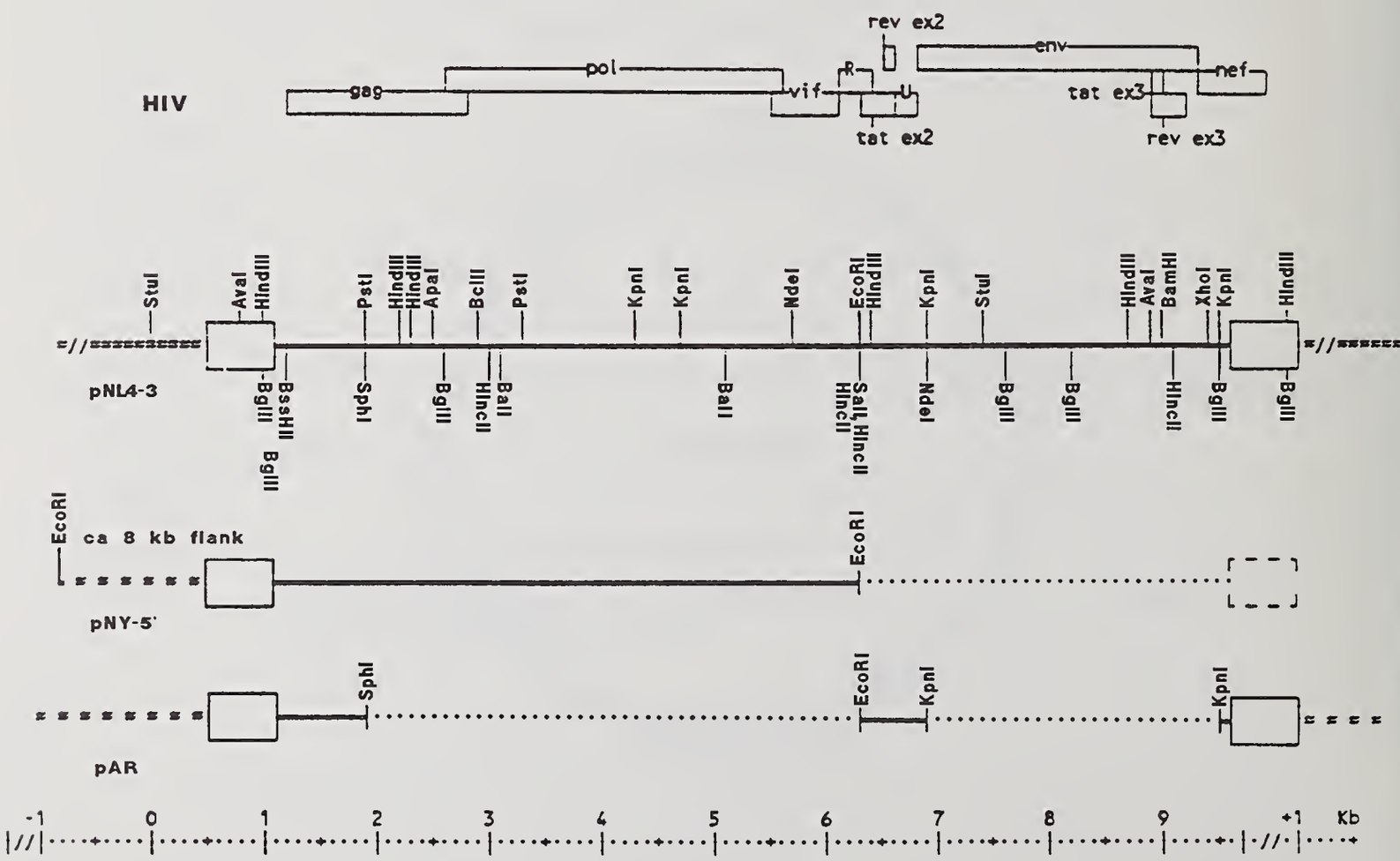
Special characteristics: *Pol* sequences are induced following removal of tryptophan from growing cultures. Induced proteins are detected optimally 2-3 hours after induction (37°C).

Contributor: Dr. Bruce Korant.

References: Ivanoff, L.A., et al. *Proc. Natl. Acad. Sci. (USA)* 83:5392, 1986.



Reagent:	**pNY-5'**
Catalog number:	345
Provided:	1 vial of transformed bacteria.
Cloning vector:	pUC18.
Bacterial host:	HB101.
Cloning site:	*Eco*RI.
Source of provirus:	A lambda clone containing *Eco*RI digested DNA extracted from cells infected with NY5 viral stock.
Description of clone:	Consists of an *Eco*RI fragment, approximately 14 kb in length, that extends from 5' flanking sequence to the *Eco*RI site at approximately 5-6 kb in the NY5 proviral sequence.
Special characteristics:	pNY-5' was used to construct the infectious HIV-1 proviral clone pNL4-3.
Contributor:	Dr. Malcolm Martin.
References:	Adachi, A., et al. *J. Virol.* **59**:284, 1986.



Reagent: **pNL4-3**

Catalog number: 114

Provided: 1 vial of transformed bacteria.

Cloning vector: pUC18.

Bacterial host: HB101.

Cloning site: *Pvu*II (pUC18), *Sma*I (5') to *Nru*I (3') fragment.

Source of provirus: NY5 (5') and LAV (3') cloned directly from genomic DNA.

Description of clone: Full length, replication and infection competent chimeric DNA. The 5' fragment of proviral NY5 (5' *Sma*I in flanking sequences to 3' *Eco*RI) and the 3' fragment of proviral LAV (5' *Eco*RI to 3' *Nru*I in flanking sequences) were blunt-end cloned into pUC18 at the *Pvu*II site after removal of polylinker sites.

Special characteristics: Upon transfection this clone directed the production of infectious virus particles in a wide variety of cells. The progeny, infectious virions, were synthesized in mouse, mink, monkey, and several non-T cell lines, indicating the absence of any intracellular obstacle to viral RNA or protein production or assembly.

Contributor: Dr. Malcolm Martin.

References: Adachi, A., et al. *J. Virol.* **59**:284, 1986.

Reagent: **pAR**

Catalog number: 344

Provided: 1 vial of transformed bacteria.

Cloning vector: pNL4-3.

Bacterial host: HB101.

Cloning site: *Sph*I- *Eco*RI, *Kpn*I.

Source of provirus: pNL4-3.

Description of clone: pNL4-3 was digested with *Sph*I and *Eco*RI, incubated with T4 polymerase, and religated. The resulting plasmid was then digested with *Kpn*I to remove proviral sequences spanning 6.3-9.0 kb. The resulting clone contains the 5' and 3'HIV-1 LTR's as well as viral sequences mapping between 5.7 and 6.3 kb.

Special characteristics: Expresses functional HIV-1 *tat* gene product (1st coding exon only) following transfection into cells.

Contributor: Dr. Malcolm Martin.

References: Personal communication.

Reagent: **pBENN 6**

Catalog number: 343

Provided: 1 vial of transformed bacteria.

Cloning vector: pBR322.

Bacterial host: HB101.

Cloning site: *Hind*III.

Source of provirus: pBENN 2 (LAV).

Description of clone: Contains the *Hind*III-*Hind*III fragment from pBENN 2 that extends from 1712 to 8188 in the LAV proviral sequence.

Special characteristics: The *Hind*III fragement can be used as a hybridization probe in Southern blots for LAV sequences.

Contributor: Dr. Malcolm Martin.

References: Folks, T., et al. *Proc. Natl. Acad. Sci. (USA)* **82**:4539, 1985.

Reagent: **pBENN 7**

Catalog number: 342

Provided: 1 vial of transformed bacteria.

Cloning vector: pBR322.

Bacterial host: HB101.

Cloning site: *Eco*RI.

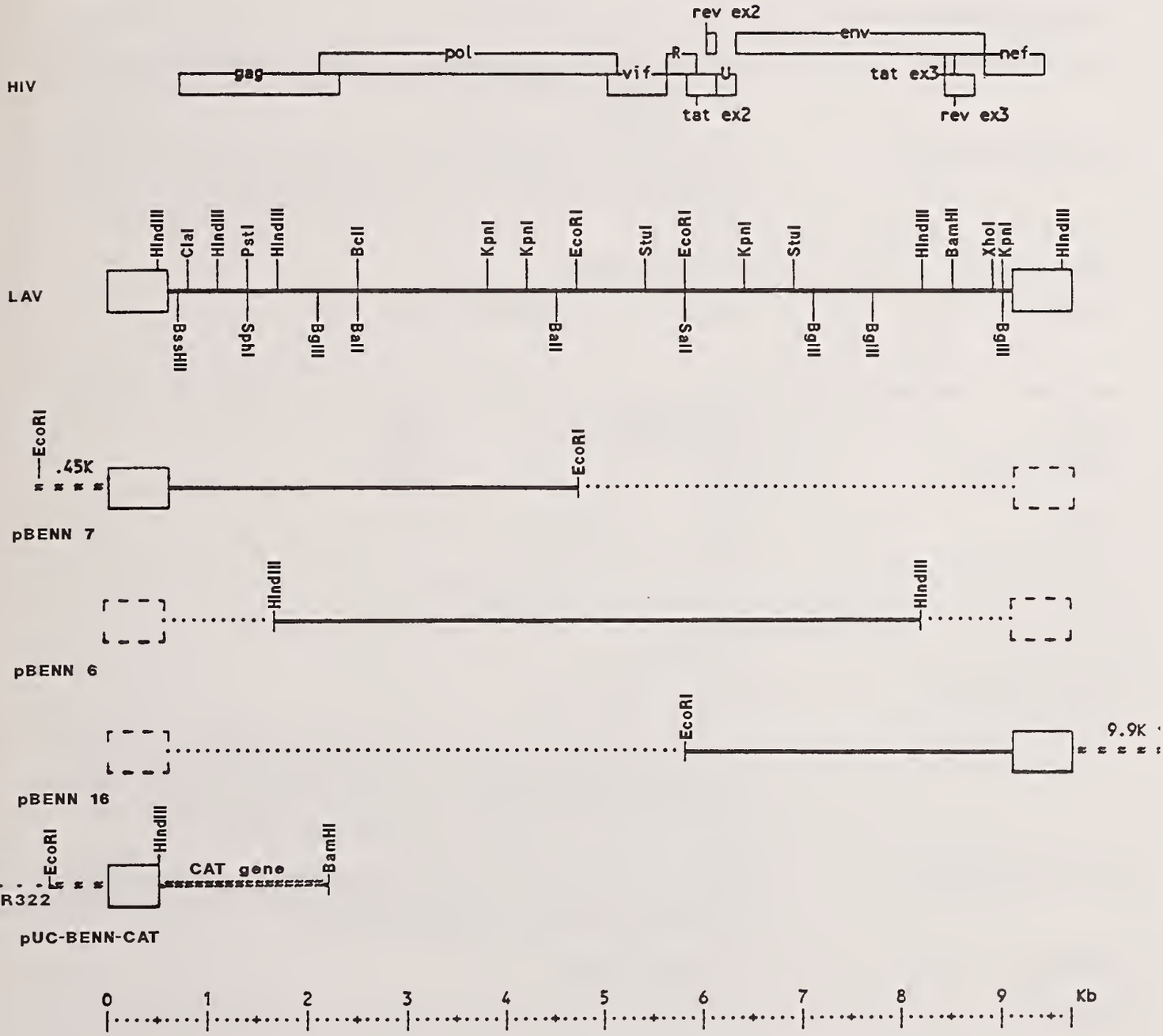
Source of provirus: pBENN 2 (LAV).

Description of clone: The 5.1 kb *Eco*RI fragment of pBENN 2 that extends from ~450 in the 5' flanking cellular sequence to 4684 in the LAV proviral sequence.

Special characteristics: This clone contains the 5' LAV LTR.

Contributor: Dr. Malcolm Martin.

References: Gendelman, H.E., et al. *Proc. Natl. Acad. Sci. (USA)* **83**:9759, 1986.


HIV
gag
pol
vif
R
U
rev ex2
tat ex2
env
nef
tat ex3
rev ex3
LAV
HindIII
ClaI
PstI
BclII
KpnI
EcoRI
StuI
BamHI
XhoI
BssHII
SphI
BglII
BalI
SalI
pBENN 7
.45K
pBENN 6
pBENN 16
9.9K
pBR322
CAT gene
pUC-BENN-CAT
0
1
2
3
4
5
6
7
8
9
Kb

Reagent: **pBENN 16**

Catalog number: 285

Provided: 1 vial of transformed bacteria.

Cloning vector: pBR322.

Bacterial host: HB101.

Cloning site: *Eco*RI.

Source of provirus: LAV.

Description of clone: Consists of an *Eco*RI fragment, ~14 kb, that extends from 5779 in the LAV proviral sequence to an *Eco*RI site about 9.9 kb in the 3' flanking cellular sequences.

Special characteristics: Subcloned from a lambda clone obtained from *Eco*RI digested DNA extract from cells infected with LAV viral stock. pBENN 16 was used in the construction of pNL4-3, an infectious clone of HIV-1 proviral DNA (Cat. #114).

Contributor: Dr. Malcolm Martin.

References: Adachi, A., et al. *J. Virol.* **59**:284, 1986.

Reagent: **pUC-BENN-CAT**

Catalog number: 481

Provided: 1 vial

Cloning vector: pUC18.

Bacterial host: HB101.

Cloning site: *Pst*I-*Bam*HI.

Source of provirus: pBENN 2 and pSV2CAT.

Description of clone: pUC-BENN-CAT resulted from the insertion of the pBR322 *Pst*I-*Bam*HI fragment (containing HIV-1 + CAT) into pUC18. The HIV-1 + CAT was a result of the ligation of the *Eco*RI-*Hind*III fragment of BENN 2 into the *Hind*III-*Bam*HI fragment of pSV2CAT. The HIV-1 + CAT was subsequently inserted into pBR322 at the *Nru*I-*Bal*I fragment.

Special characteristics: pBENN-CAT contains a bacterial chloramphenicol acetyl transferase (CAT) gene driven by an HIV-1 (LAV) LTR promoter. The pUC-BENN-CAT plasmid grows better than prior constructs.

Contributor: Dr. Malcolm Martin.

References: Gendelman, H.E., et al. *Proc. Natl. Acad. Sci. (USA)* **83**:9759, 1986.

Reagent: **pBKBH10S**

Catalog number: 182

Provided: 1 vial of transformed bacteria.

Cloning vector: pBluescript $M13^+$ with KS polylinker.

Bacterial host: JM109.

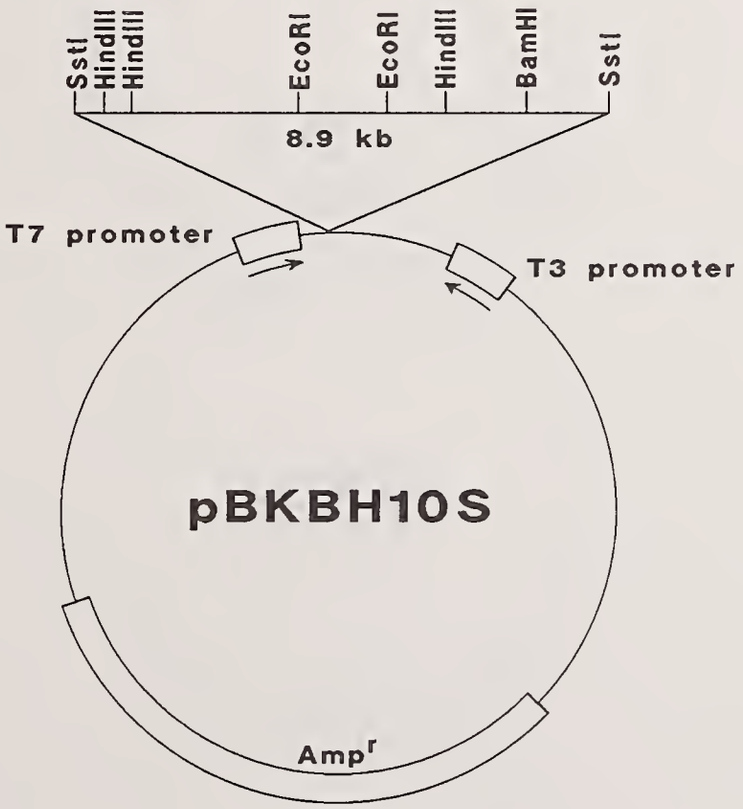
Cloning site: *Sst*I-*Sst*I.

Description of clone: Contains the 8.9 kb *Sst*I (222) to *Sst*I (9154) fragment of pBH10-R3.

Special characteristics: Contains all HIV-1 gene coding regions but does not contain HIV-1 LTR.

Contributor: Dr. John Rossi.

References: Personal communication.



Reagent: **pGM91 (pGM93)**

Catalog number: 183

Provided: 1 vial of transformed bacteria.

Cloning vector: pGEM-2.

Bacterial host: JM109.

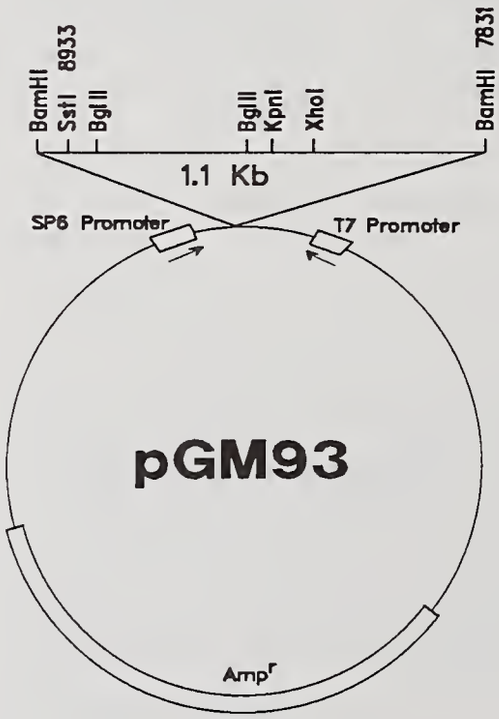
Cloning site: *Bam*HI.

Description of clone: 1.1 kb *Bam*HI (8053)-*Sst*I (9154) fragment of pBH10-R3 but excised using *Bam*HI (8053) site on pBH10-R3 and *Bam*HI site in the polylinker pBH10-R3 and cloned as *Bam*HI fragment in pGEM-2.

Special characteristics: Contains nearly the entire 3' exon of the *nef* gene including the complete coding sequence of the Nef protein.

Contributor: Dr. John Rossi.

References: Murkawa, G.J., et al. *DNA* 7:287, 1988.



Reagent: **pGM92**

Catalog number: 184

Provided: 1 vial of transformed bacteria.

Cloning vector: pGEM-2.

Bacterial host: JM109.

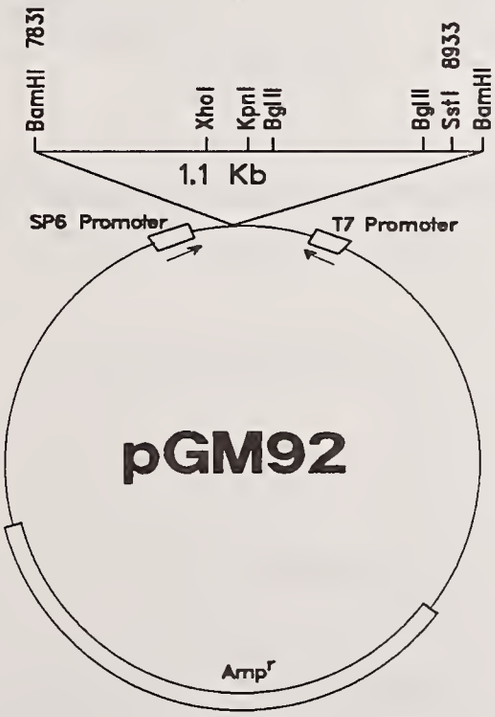
Cloning site: *Bam*HI.

Description of clone: Same as in pGM91 but in the opposite orientation. Transcription using SP6 RNA polymerase will give sense RNA and transcription using T7 will give antisense RNA.

Special characteristics: Contains nearly the entire 3' exon of the *nef* gene, including the complete coding sequence of 3' Rev protein.

Contributor: Dr. John Rossi.

References: Murkawa, G.J., et al. *DNA* 7:287, 1988.



Human Immunodeficiency Virus 2

Reagent: **HIV-2$_{ROD}$ phage**

Catalog number: 207

Provided: 1 vial of transformed bactéria.

Cloning vector: λEMBL3.

Bacterial host: Phage stock (last grown on LE392).

Cloning site: *Bgl*II-*Bam*HI.

Source of provirus: HIV-2$_{ROD}$ producing CEM cells provided by Dr. L. Montagnier.

Description of clone: *Bgl*II-digested total cell DNA from CEM-HIV-2$_{ROD}$ was inserted into the *Bam*HI site of λEMBL3 to form a library, which was then screened with pK2 *Bam*A to obtain full-length molecular clones.

Contributor: Dr. Ronald Desrosiers.

References: Naidu, Y.M., et al. *J. Virol.* **62**:4691, 1988.

HIV-2$_{ROD}$

Reagent: **pJSP4-27/H6**

Catalog number: 181

Provided: 1 vial of transformed bacteria.

Cloning vector: pTZ-18.

Bacterial host: TG-1.

Cloning site: *Hind*III.

Source of provirus: Senegalese HIV-2 isolate (HIV-2$_{ST}$) with attenuated *in vitro* cytopathicity.

Description of clone: Clone pJSP4-27/H6 represents a 6 kb *Hind*III fragment of λJSP4-27 subcloned into pTZ-18. The insert contains part of the HIV-2$_{ST}$ *vpr* gene, complete *tat*, *rev*, *env*, and *nef* ORFs, the complete LTR, and 3' flanking cellular sequences.

Special characteristics: This *Hind*III subclone of λJSP4-27 comprises the 3' portion of the HIV-2$_{ST}$ provirus plus flanking cellular sequences.

Contributor: Dr. Beatrice Hahn and Dr. George M. Shaw.

References: Kong, L.I., et al. *Science* **240**:1525, 1988.

Simian Immunodeficiency Virus

Reagent: **$SIV_{mac}239$ phage**

Catalog number: 210

Provided: 1 vial of transformed bacteria.

Cloning vector: λEMBL4.

Bacterial host: Phage stock (last grown on LE392). Contributor uses NM539.

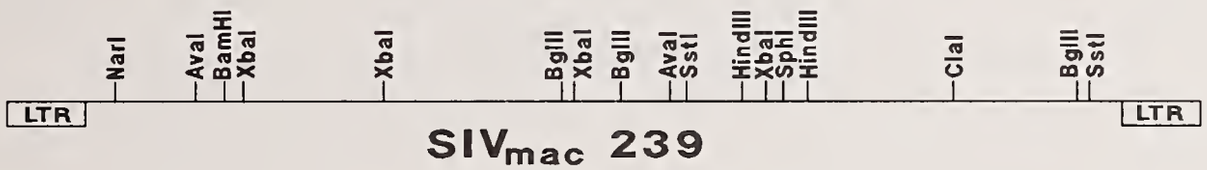
Cloning site: *Eco*RI.

Source of provirus: Integrated copy from HUT 78 grown virus.

Description of clone: *Eco*RI-digested total cell DNA from HUT 78-$SIV_{mac}239$ was inserted into the *Eco*RI site of λEMBL4 to create a library, which was then screened with pK2 *Bam*A to obtain full-length molecular clones.

Contributor: Dr. Ronald Desrosiers.

References: Naidu, Y.M., et al. *J. Virol.* **62**:4691, 1988.



Reagent: **$SIV_{mac}142$ phage**

Catalog number: 211

Provided: 1 vial of transformed bacteria.

Cloning vector: λEMBL3.

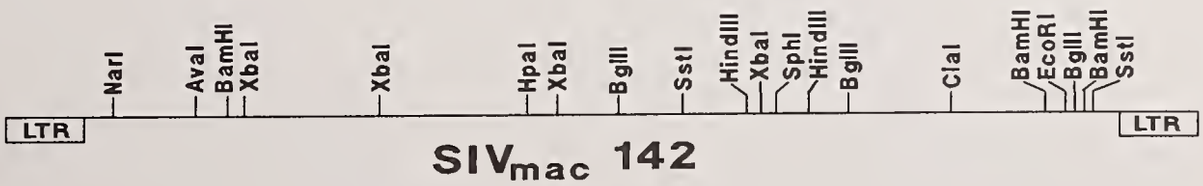
Bacterial host: Phage stock (last grown on LE392). Contributor uses NM539.

Cloning site: *Sau*3A partial-*Bam*HI.

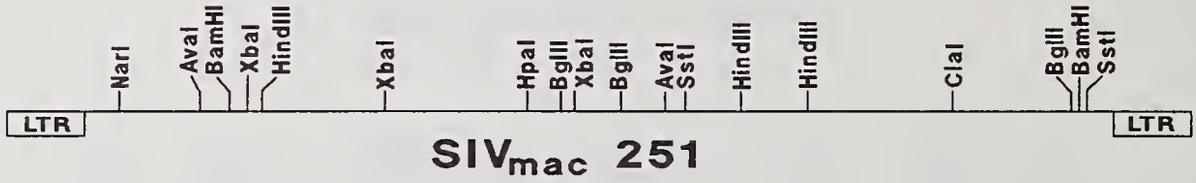
Source of provirus: Integrated copy of HUT 78 grown virus.

Contributor: Dr. Ronald Desrosiers.

References: Naidu, Y.M., et al. *J. Virol.* **62**:4691, 1988.



Reagent:	**$SIV_{mac}251$ phage**
Catalog number:	213
Provided:	1 vial of transformed bacteria.
Cloning vector:	λEMBL 4.
Bacterial host:	Phage stock (last grown on LE392). Contributor uses NM539.
Cloning site:	*Eco*RI.
Source of provirus:	Integrated copy from HUT 78 grown virus.
Description of clone:	*Eco*RI-digested total cell DNA of HUT 78-$SIV_{mac}251$ was inserted into the *Eco*RI site of λEMBL4 to form a library, which was then screened with pK2 *Bam*A to obtain full-length molecular clones.
Contributor:	Dr. Ronald Desrosiers.
References:	Naidu, Y.M., et al. *J. Virol.* **62**:4691, 1988.



SIV_{mac} 251

Reagent: **pBK28-SIV**

Catalog number: 133

Provided: 1 vial of transformed bacteria.

Cloning vector: pUC18.

Bacterial host: JM109.

Cloning site: *Eco*RI-*Eco*RV into *Eco*RI-*Hinc*II sites.

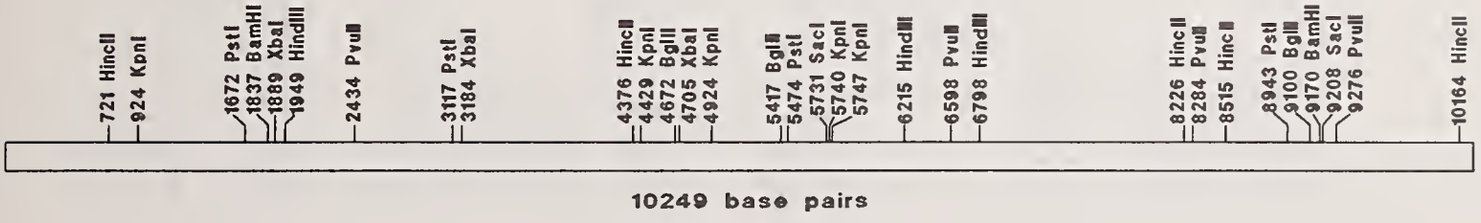
Source of provirus: pK289 cells, originally thought to contain HTLV-4, now known to be derived from SIV_{mac} isolate 251 from New England Regional Primate Center.

Special characteristics: Difficult to propagate in *E. coli* without deletions. This preparation should grow without problems when bacteria are kept at room temperature and the culture is grown to $A_{600} \leq 0.5$. It is an infectious molecular clone, which gives rise to persistent infection and lymphadenopathy in rhesus macaques. The virus failed to induce acute onset immunodeficiency disease in four macaques within 1 year of inoculation. One animal died 17 months post-inoculation following anemia and later, renal failure; the rest are healthy and infected as of 21 months. HUT 78 cells infected with BK-28 (catalog # 173) are also available.

Contributor: Dr. James I. Mullins.

References: Kornfield, H., et al. *Nature* **326**:610, 1987.

NOTE: *Genbank Locus Name: HIVHTLV4A,SIVMM251; Accession Number: Y00269, XO6393.*



pBK28-SIV

Feline Leukemia Virus

Reagent: **pEECC - FeLV**

Catalog number: 105

Provided: 1 vial of transformed bacteria.

Cloning vector: pUC18.

Bacterial host: JM109.

Cloning site: 5' *Eco*RI-3' *Sma*I (non-functional).

Source of provirus: 61E and 61C cloned directly from intestinal tissue of cat 1161 which had been inoculated with FeLV-FAIDS strain and developed fatal immunodeficiency disease.

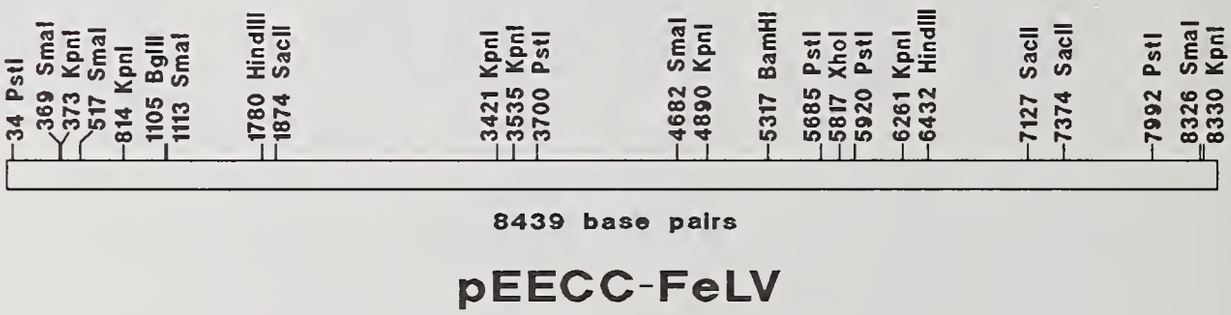
Description of clone: Full length, replication competent chimeric virus containing 61E-FeLV derived *gag*, *pol*, and R/U5 (LTR) sequences; and 61C derived *env*, U3 (LTR) sequences.

Special characteristics: Infectious molecular clone, T cell cytopathic *in vitro*, induces immunodeficiency disease *in vivo*. AH927 infected with EECC (catalog #170) is also available.

Contributor: Dr. James I. Mullins.

References: Overbaugh, J., et al. *Science* **239**:906, 1988.

NOTE: *Genbank Locus Name: FCVRD; Accession Number: M18246.*



Reagent: **p61E - FeLV**

Catalog number: 109

Provided: 1 vial of transformed bacteria.

Cloning vector: pUC18.

Bacterial host: JM109.

Cloning site: *Eco*RI.

Source of provirus: From a λgtWesλB library of DNA from intestine of cat 1161 that had been inoculated with FeLV-FAIDS strain and developed fatal immunodeficiency disease.

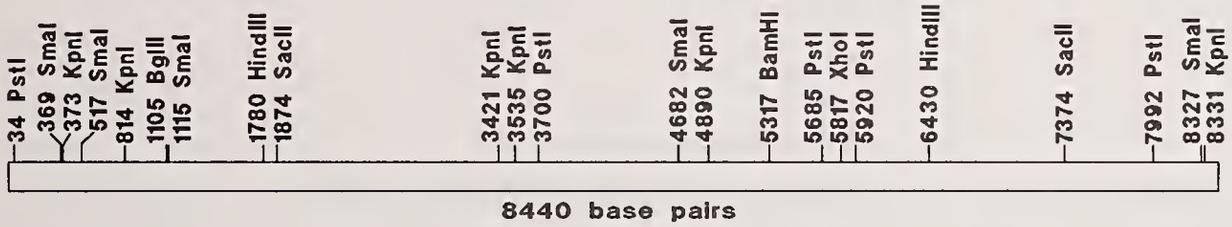
Description of clone: Represents full length, replication competent FeLV. Includes flanking cat genomic DNA.

Special characteristics: Infectious and minimally pathogenic when inoculated into specific pathogen free cats. AH927 cells infected with 61E (catalog #168) are also available.

Contributor: Dr. James I. Mullins.

References: Donahue, P.R., et al. *J. Virol.* **62**:722, 1988.
Overbaugh, J., et al. *Science* **239**:906, 1988.

NOTE: *Genbank Locus Name: FCVF6A; Accession Number: M18247.*



p61E-FeLV

Other

Reagent: **pH6 B 5.0**
Catalog number: 396
Provided: 1 vial of transformed bacteria.
Cloning vector: pBR322.
Bacterial host: HB101.
Cloning site: *Bam*HI.
Source of provirus: Lambda H6 from Mo-T cells (HTLV-II Mo).
Description of clone: Contains HTLV-II DNA from *Bam*HI site at nucleotide 361 in the LTR to *Bam*HI site 5090 in *pol*. Contains amp^r marker.
Contributor: Dr. Irvin S.Y. Chen.
References: Chen, I.S.Y., et al. *Nature* **305**:502, 1983.

Reagent: **pH6 B 3.5**
Catalog number: 397
Provided: 1 vial of transformed bacteria.
Cloning vector: pBR322.
Bacterial host: HB101.
Cloning site: *Bam*HI.
Source of provirus: Lambda H6 from Mo-T cells (HTLV-II Mo).
Description of clone: Contains HTLV-II DNA from *Bam*HI site at nucleotide 5090 through nucleotide 8550. Contains amp^r marker.
Contributor: Dr. Irvin S.Y. Chen.
References: Chen, I.S.Y., et al. *Nature* **305**:502, 1983.

Reagent: pT4B

Catalog number: 157

Provided: 1 vial of transformed bacteria.

Cloning vector: SP65.

Bacterial host: HB101.

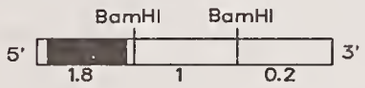
Cloning site: *Eco*RI.

Description of clone: The cDNA insert is 3.0 kb, encoding the CD4 receptor of human T lymphocytes of which 1.5 kb is the coding sequence. Contains amp^r marker.

Special characteristics: When placed in expression vectors and after transformation the cDNA converts $CD4^-$ fibroblasts to the $CD4^+$ phenotype.

Contributor: Dr. Richard Axel.

References: Maddon, P.J., et al. *Cell* **42**:93, 1985.



T4B cDNA
(3 Kb)

Reagent: pT8F1

Catalog number: 179

Provided: 1 vial of transformed bacteria.

Cloning vector: SP65.

Bacterial host: HB101.

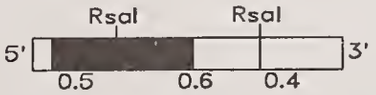
Cloning site: *Eco*RI.

Description of clone: The cDNA insert is 1.5 kb encoding the CD8 receptor of human peripheral CD8 lymphocytes of which 0.7 kb is the coding sequence. Contains amp^r marker.

Special Characteristics: When placed in expression vectors and after transformation the cDNA converts $CD8^-$ fibroblasts to the $CD8^+$ phenotype.

Contributor: Dr. Richard Axel.

References: Littman, D.R., et al. *Cell* 40:237, 1985.



T8F1 cDNA
(1.5 Kb)

EXPRESSION SYSTEMS

Prepared in Bacteria/Expressed in Bacteria

Reagent: **pKRT2 (HIV-1 RT)**

Catalog number: 393

Provided: 1 vial of transformed bacteria.

Cloning vector: pKK233-2.

Host: JM105. The contributor also uses JM109.

Cloning site: *Nco*I-*Hind*III.

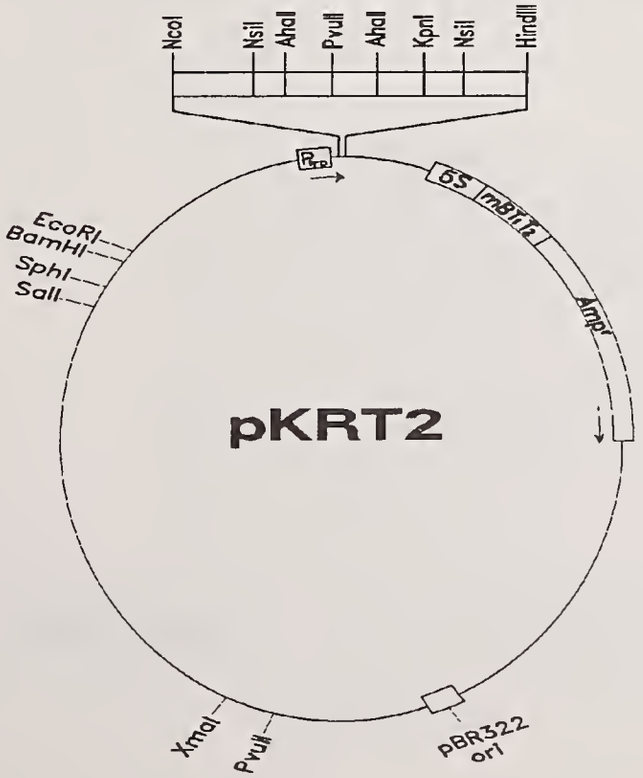
Cloning strategy: *In vitro* mutagenesis was done on clone BH10-derived DNA to introduce translational initiation codons, as well as restriction sites to facilitate cloning, at points corresponding to sites of cleavage of RT from *gag/pol* precursor by the HIV-1 protease.

Description of clone: High level expression of unfused HIV-1 RT that differs from the native virion reverse transcriptase by only one amino acid (the N-terminal proline is changed to alanine). Expression occurs from the plasmid's *trc* promoter.

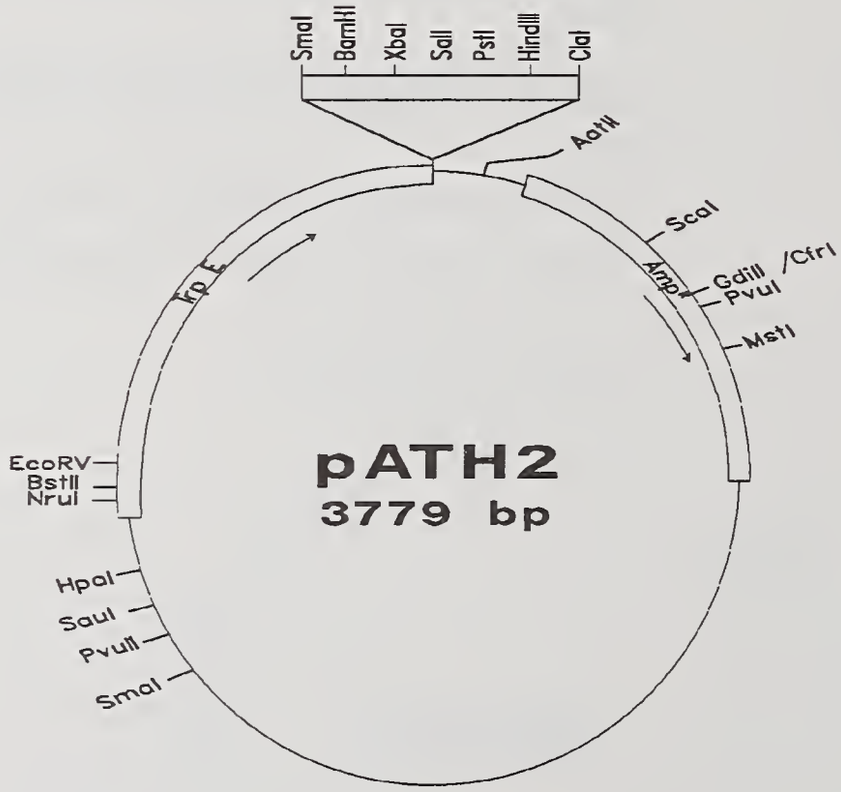
Special characteristics: The HIV-1 RT produced from this expression plasmid is functional as both an RNA-dependent DNA polymerase and a ribonuclease H in *E. coli* extracts.

Contributor: Dr. Richard D'Aquila and Dr. William C. Summers.

References: D'Aquila, R.T and Summers, W.C. *J. Acq. Imm. Def. Syn.* 2:579, 1989.



Reagent:	**pATH2**
Catalog number:	186
Provided:	1 ml of transformed bacteria.
Cloning vector:	pBR322.
Host:	HB101.
Cloning site:	Polylinker, many sites.
Description of clone:	Expression vector.
Special characteristics:	Expresses inserted sequences as *trpE*-X fusions, under control of the *trp* promoter.
Contributor:	Dr. Stephen Goff.
References:	Spindler, K.R., Fosser, D.S.E., and Berk, A.J. *J. Virol.* **49**:132, 1984.



Reagent: **pHRT25**

Catalog number: 63

Provided: 1 vial of transformed bacteria.

Cloning vector: pATH2.

Host: HB101.

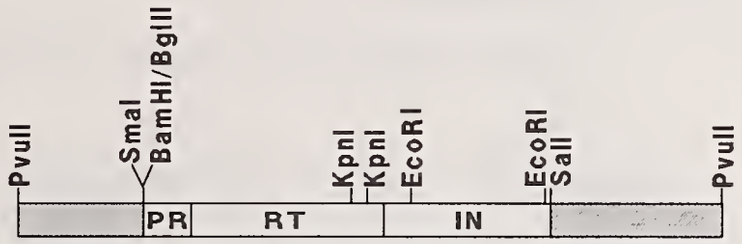
Cloning site: *Bam*HI(nonfunctional)-*Sal*I.

Description of clone: pHRT25 encodes a *trpE-pol* fusion with almost all the HIV-1 *pol* region. Contains amp^r marker.

Special characteristics: Encodes a functional reverse transcriptase.

Contributor: Dr. Stephen Goff.

References: Tanese, N., et al. *J. Virol.* **59**:743, 1986.



pHRT25

Reagent: **p22K56**

Catalog number: 65

Provided: 1 vial of transformed bacteria.

Cloning vector: pATH2.

Host: HB101.

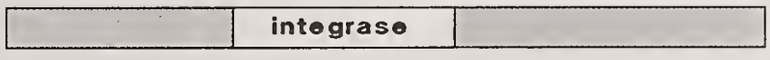
Cloning site: Initially a *Bgl*II-*Sal*I HIV-1 *pol* fragment was cloned into *Bam*HI-*Sal*I sites of pATH2 (pHRT22). This construct was linearized with *Kpn*I, subjected to *Bal*31 digestion followed by digestion with *Sma*I and reclosure of the large fragment.

Description of clone: Encodes *trpE*-HIV-1 integration function protein.

Special characteristics: p22K56 encodes a *trpE*-pol fusion with only the HIV-1 integrase region. Derived from pHRT25, it has the protease and RT domains removed.

Contributor: Dr. Stephen Goff.

References: Personal communication.



p22K56

Reagent: **pRX3B2 (pHRTRX2)**

Catalog number: 64

Provided: 1 vial of transformed bacteria.

Cloning vector: pATH2.

Host: HB101.

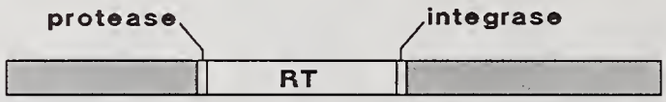
Cloning site: Originally in *Bam*HI/*Sal*I sites of pATH2, but subsequent modifications to remove the protease and integrase regions (which include exonucleolytic digestion at both ends) have resulted in loss of both these sites.

Description of clone: Encodes *trpE*-HIV-1 RT fusion protein of approximately 100 kD. Displays both DNA polymerase and RNase H activities.

Special characteristics: Makes a stable protein which displays both DNA polymerase and RNase H functions of reverse transcriptase.

Contributor: Dr. Stephen Goff.

References: Tanese, N., Prasad, V.R., and Goff, S.P. *DNA* 7:407, 1988.



pHRTRX2

Reagent: **pBRT1*prt*$^+$**

Catalog number: 128

Provided: 50 μl of transformed bacteria.

Cloning vector: Phagemid from contributor's laboratory.

Host: JM101.

Cloning site: *Bgl*II/*Eco*RI.

Description of clone: Plasmid contains a *Bgl*II to *Eco*RI fragment with the protease and reverse transcriptase coding domains of pBENN 2 inserted between the *Bam*HI and *Eco*RI sites in the pIBI21 polylinker region.

Special characteristics: This plasmid expresses protease and RT in bacterial cells.

Contributor: Dr. Ronald Swanstrom.

References: Farmerie, W.G., et al. *Science* **236**:305, 1987.

NOTE: ***Complete transfection protocol is included with each shipment.***

Reagent: pBRT3*prt*$^-$

Catalog number: 129

Provided: 50 μl of transformed bacteria.

Cloning vector: Phagemid from contributor's laboratory.

Host: JM101.

Cloning site: *Hinc*II-*Eco*RI.

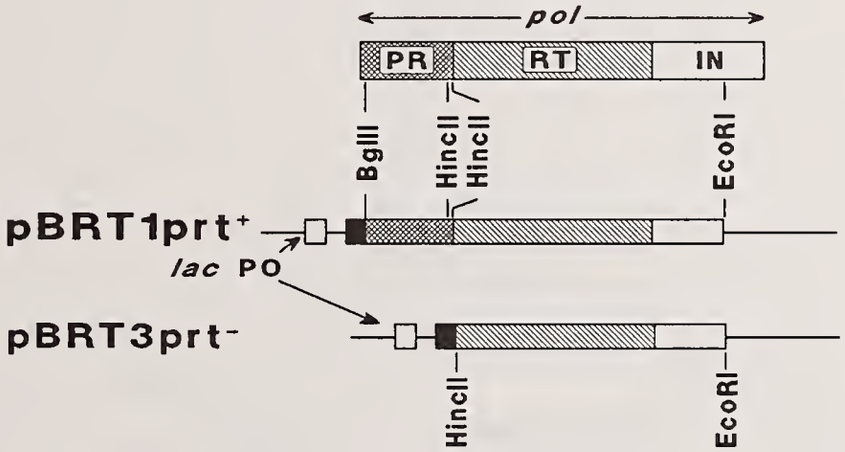
Description of clone: Plasmid contains a *Hinc*II to *Eco*RI fragment of pBENN 2 inserted between the *Hinc*II and *Eco*RI sites in the pIBI21 polylinker region.

Special characteristics: This plasmid can serve as a control for pBRT1*prt*$^+$ as it does not express the protease domain.

Contributor: Dr. Ronald Swanstrom.

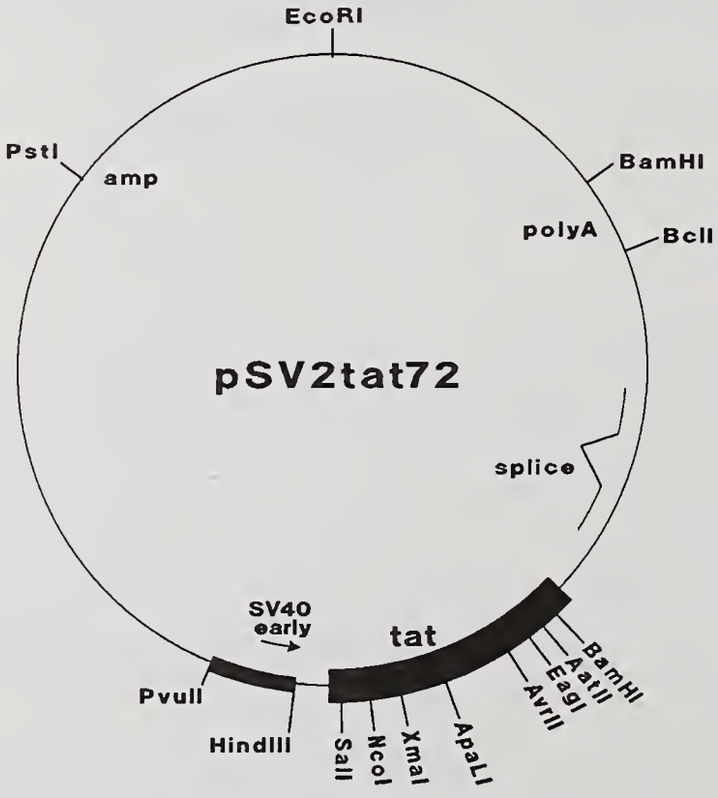
References: Famerie, W.G., et al. *Science* **236**:305, 1987.

NOTE: ***Complete transfection protocol is included with each shipment.***



Prepared in Bacteria/Expressed in Cells

Reagent:	**pSV2*tat*72**
Catalog number:	294
Provided:	1 vial of transformed bacteria.
Cloning vector:	pSV2-*dhfr*.
Host:	HB 101. Can be grown in most strains of *E. coli*.
Cloning site:	See description below.
Description of clone:	Produces Tat (residues 1-72) using the SV40 early promoter. Constructed by replacing the *dhfr* gene in pSV2-*dhfr* with a synthetic gene encoding Tat.
Special characteristics:	Contact contributor for additional information.
Contributor:	Dr. Alan Frankel.
References:	Frankel, A.D. and Pabo, C.O. *Cell* **55**:1189, 1988.



Reagent: pHIV*lacZ*

Catalog number: 151

Provided: 1 vial of transformed bacteria.

Cloning vector: pU3RIII.

Host: HB101.

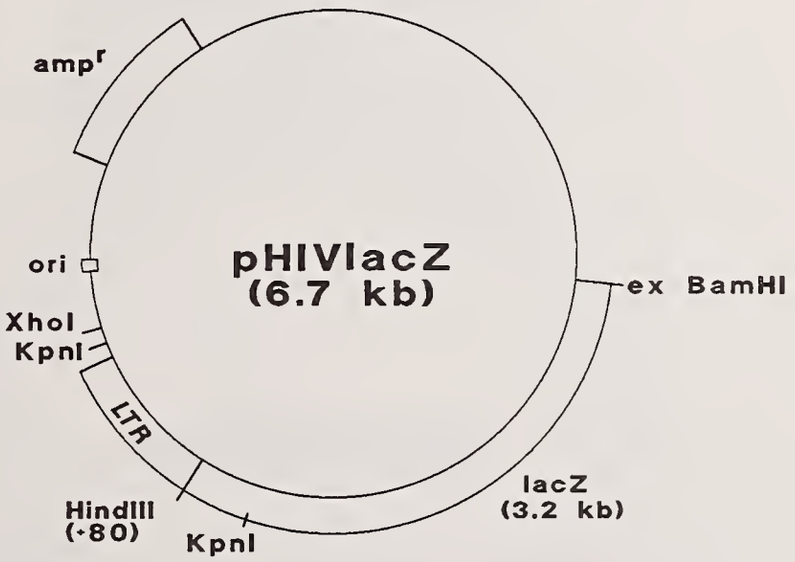
Cloning site: *Hind*III-*Bam*HI.

Description of clone: The plasmid contains the HIV-1 3' LTR driving the *E. coli lacZ* gene.

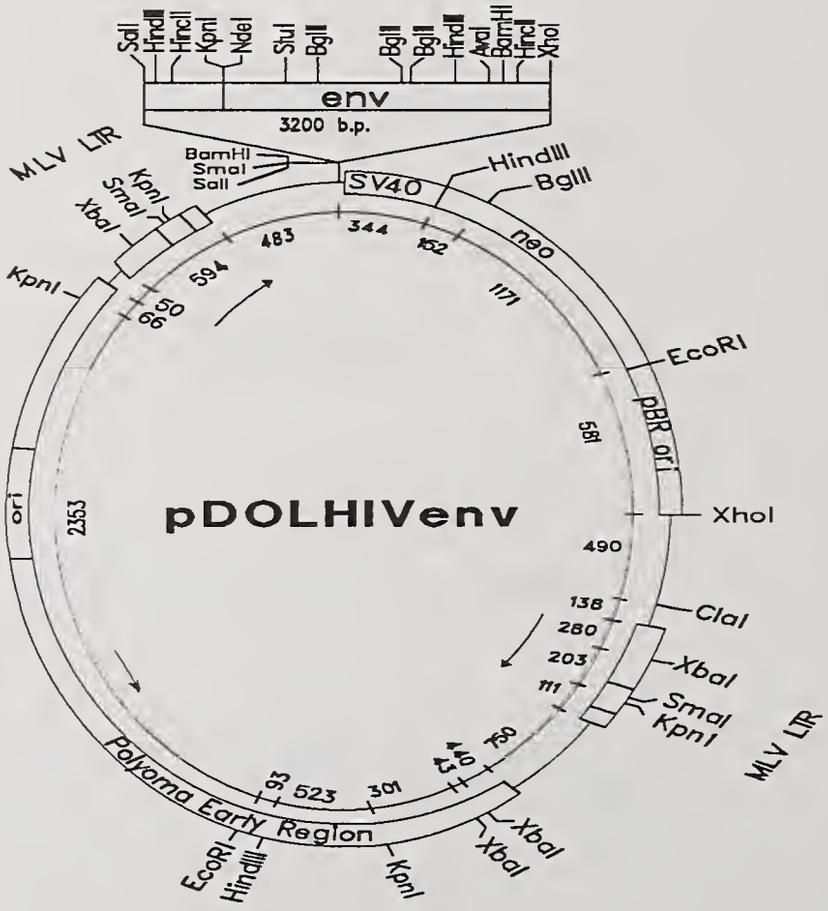
Special characteristics: Standard β-galactosidase assays show quite high levels of expression in human embryonic teratocarcinoma cells or activated monocyte-macrophage lines. Contains no *Bam*HI site.

Contributor: Dr. Joseph J. Maio.

References: Maio, J.J. and Brown, F.L. *J. Virol.* **62**:1398, 1988.



Reagent:	**pDOLHIV*env***
Catalog number:	324
Provided:	1 vial of transformed bacteria.
Cloning vector:	pDOL.
Host:	JM105. The contributor also uses JM107.
Cloning site:	*Sal*I.
Cloning strategy:	The *Sal*I-*Xho*I region of pNL4-3 was introduced into the *Sal*I site of pDOL.
Description of clone:	pDOLHIV*env* contains the open reading frames for the *env, tat,* and *rev* coding regions as the pNL4-3 insert.
Special characteristics:	This construct efficiently expresses envelope glycoproteins when transfected into HeLa T4 cells. These transfected cells form syncytia indistiguishable from those formed by HeLa T4 infected with HIV-1.
Contributor:	Eric O. Freed and Dr. Rex Risser.
References:	Freed, E.O., Myers, D.J., and Risser, R. *J. Virol.* **63**:4670, 1989.



Reagent: **pDOLHIV*env*R**

Catalog number: 322

Provided: 1 vial of transformed bacteria.

Cloning vector: pDol.

Host: JM105. Contributor also uses JM107.

Cloning site: *Sal*I.

Cloning strategy: The *Sal*I-*Xho*I region of pNL4-3 was introduced into the *Sal*I site of pDOL in an orientation inverted to that of pDOLHIV*env*.

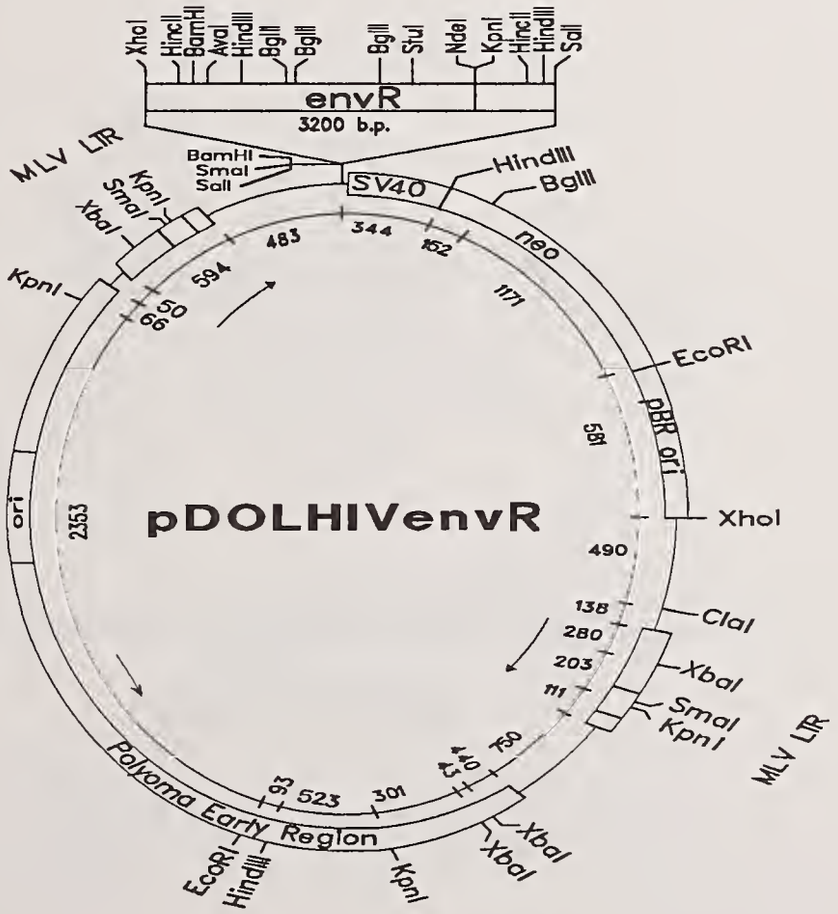
Description of clone: pDOLHIV*env*R contains the open reading frames for the *env, tat,* and *rev* coding regions in an orientation inverted to that of pDOLHIV*env*.

Special characteristics: This construct does not express envelope proteins when transfected into HeLa T4 cells, nor does transfection result in syncytia formation.

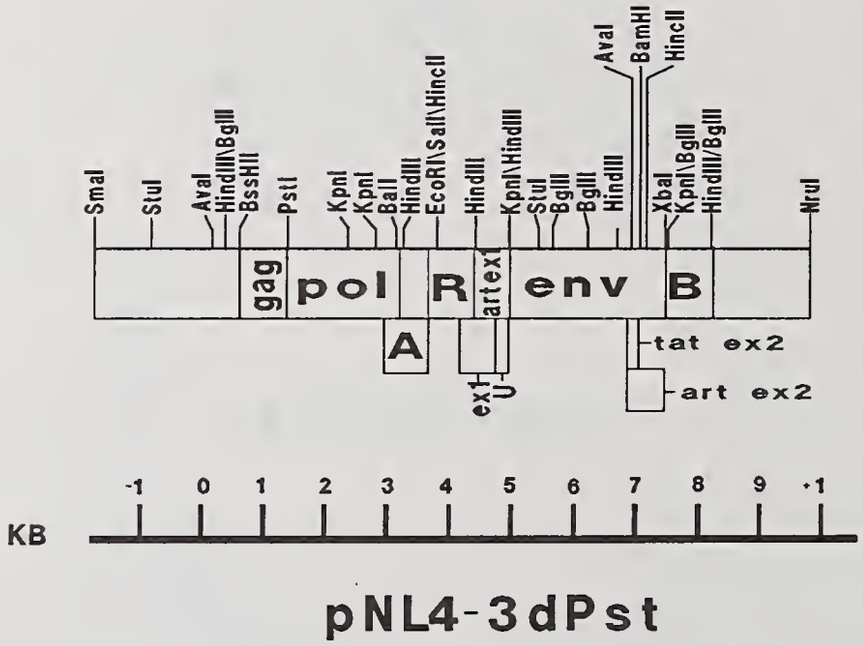
Contributor: Eric O. Freed and Dr. Rex Risser.

References: Freed, E.O., Myers, D.J., and Risser, R. *J. Virol.* 63:4670, 1989.

NOTE: *This reagent serves as a control to catalog numbers 323, 324, and 513.*



Reagent:	**pNL4-3d*Pst***
Catalog number:	323
Provided:	1 vial of transformed bacteria.
Cloning vector:	pNL4-3.
Host:	JM105. The contributor also uses JM107.
Cloning site:	*Pst*I.
Cloning strategy:	The region from *Pst*I in the *gag* coding region to *Pst*I in the *pol* coding region of pNL4-3 was removed.
Description of clone:	pNL4-3 is a *gag/pol* deletion mutant.
Special characteristics:	This construct efficiently expresses envelope glycoproteins when transfected into HeLa T4 cells. These transfected cells form syncytia indistinguishable from those formed by HeLa T4 infected with HIV-1.
Contributor:	Eric O. Freed and Dr. Rex Risser.
References:	Freed, E.O., Myers, D.J., and Risser, R. *J. Virol.* **63**:4670, 1989.



pNL4-3dPst

Reagent: pH*env*

Catalog number: 513

Provided: 1 vial of transformed bacteria.

Cloning vector: pIII*env*3-1.

Host: JM105. Contributor also uses JM107.

Cloning site: *Sal*I-*Bam*HI.

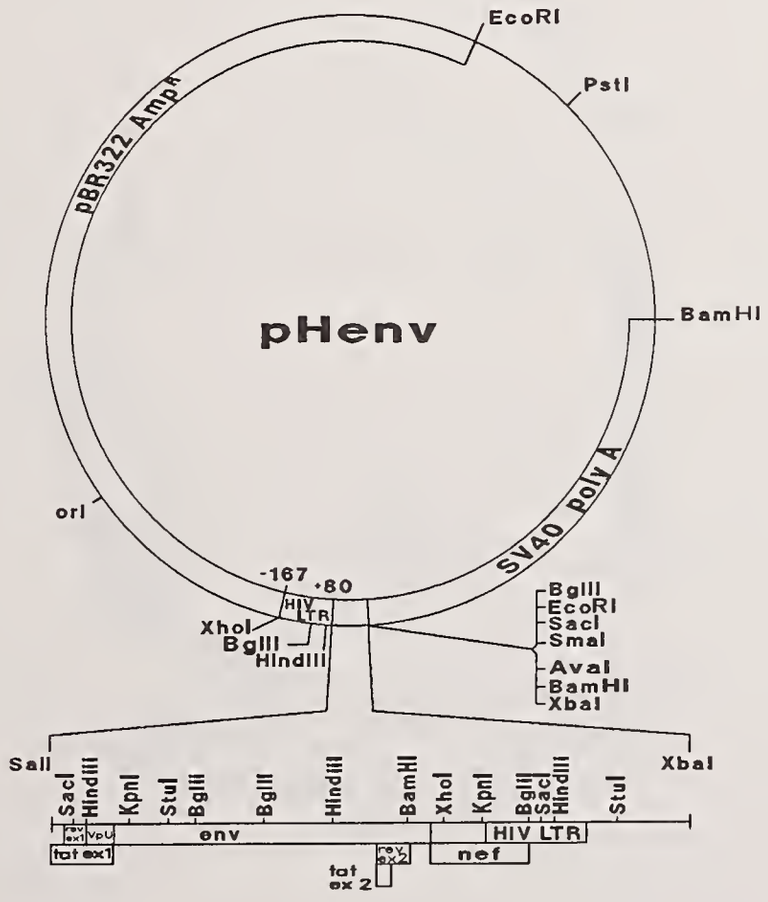
Cloning strategy: The *Sal*I-*Bam*HI region of pIII*env*3-1 containing the mutant *tat* sequence was replaced by the *Sal*I-*Bam*HI region of pNL4-3.

Description of clone: This clone contains a restored *tat* open reading frame as well as the HIV-1 long terminal repeat immediately 5' to the *env*, *tat*, and *rev* coding regions.

Special characteristics: This construct efficiently expresses envelope glycoproteins when transfected into HeLa T4 cells. These transfected cells form syncytia indistinguishable from those formed by HeLa T4 infected with HIV-1.

Contributor: Eric O. Freed and Dr. Rex Risser.

References: Freed, E.O., Myers, D.J., and Risser, R. *J. Virol.* **63**:4670, 1989.



Reagent: **pIII*env*3-1**

Catalog number: 289

Provided: 1 vial of transformed bacteria.

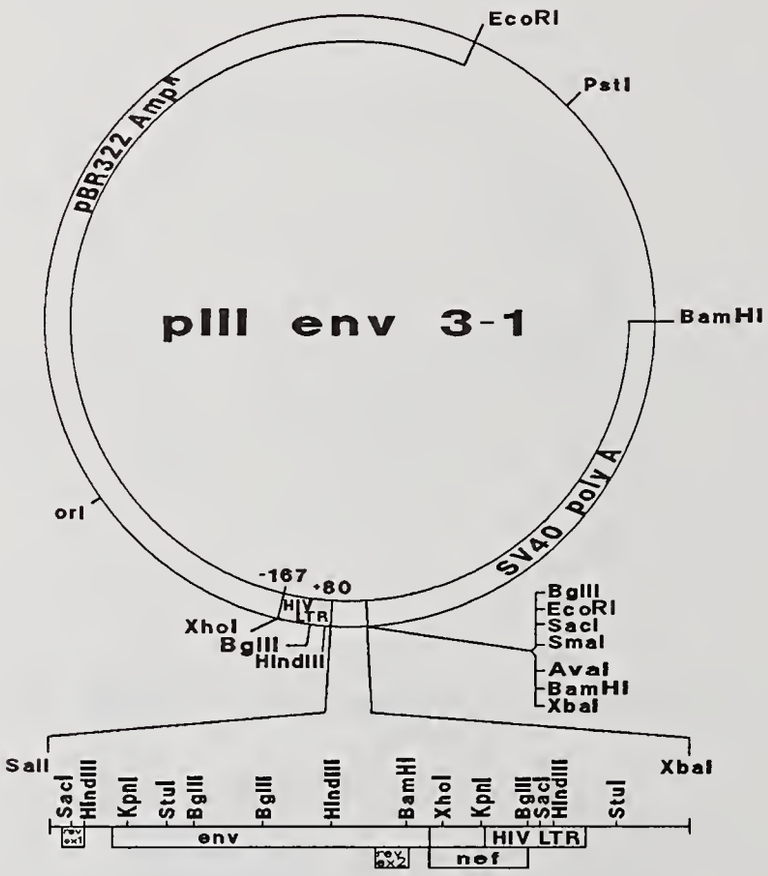
Cloning vector: pBR322.

Host: HB101.

Description of clone: The Tat-responsive HIV-1 LTR is used to promote expression of the HIV-1 (HXB2) *rev* and *env* genes. The plasmid contains a fragment of the HXB2 provirus from 5496 (an artificial *Sal*I site) 3' to the 3' terminal LTR.

Contributor: Dr. Joseph Sodroski.

References: Sodroski, J., et al. *Nature* **322**:470, 1986.



Reagent: **pU3R-III CAT**

Catalog number: 330

Provided: 1 vial of transformed bacteria.

Cloning vector: pSV$_2$CAT.

Host: HB101.

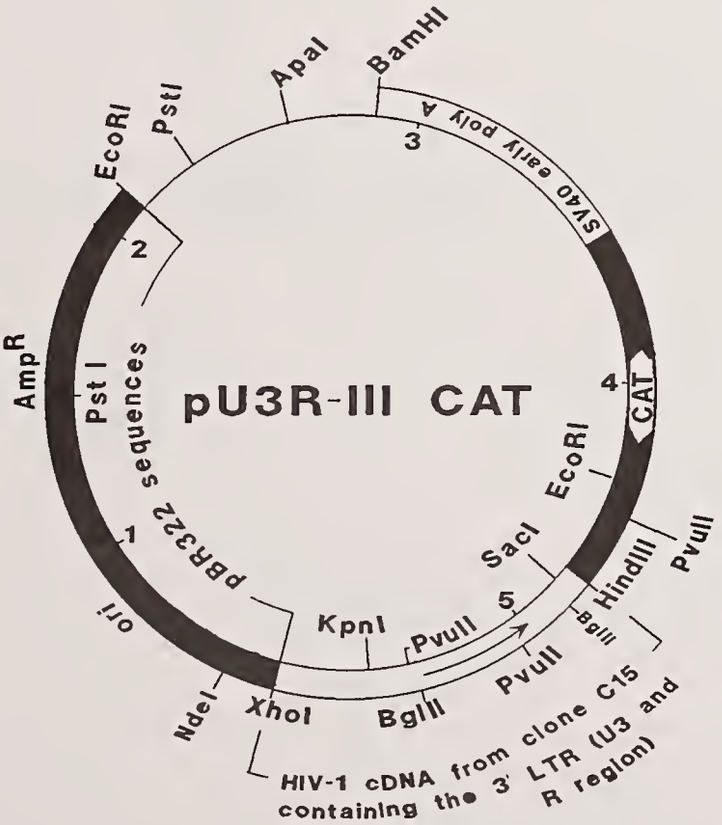
Cloning site: *Xho*I-*Hind*III.

Description of clone: *Xho*I-*Hind*III fragment (~ 720 base pairs) of an HIV-1 cDNA containing the U3 and R regions of the 3' LTR cloned 5' to the chloramphenicol acetyltransferase (CAT) gene.

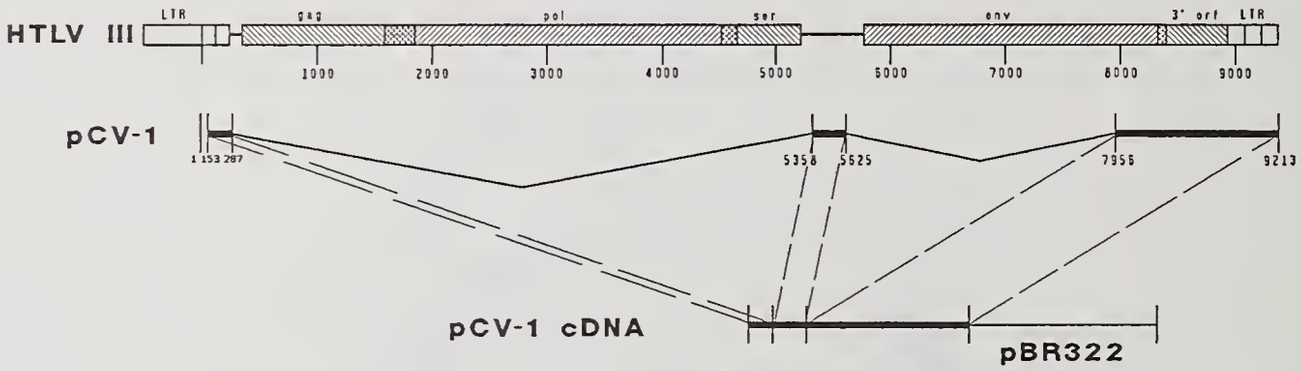
Special characteristics: This plasmid will direct the expression of CAT under control of the HIV-1 LTR sequences that are responsive to Tat.

Contributor: Dr. Joseph Sodroski.

References: Rosen, C.A., et al. *J. Virol.* **57**:379, 1986.
Sodroski, J., et al. *Science* **227**:171, 1985.



Reagent:	**pCV1**
Catalog number:	303
Provided:	1 vial of transformed bacteria.
Cloning vector:	pCV, a mammalian expression vector containing hybrid regulatory sequences, size 7.0 kb.
Host:	HB101.
Cloning site:	*PstI*.
Description of clone:	Contains 1.5 kb of pBR322 sequences adjacent to cDNA sequences. Insert size approximately 1.8 kb. It encodes both Tat and Rev.
Special characteristics:	Tetracycline resistant. *Pst*I cut gives two fragments of 3.3 kb and 7.0 kb. 3.3 kb = 1.8 + 1.5 kb.
Contributor:	Dr. Flossie Wong-Staal.
References:	Arya, S.K., et al. *Science* **229**:69, 1985.



Reagent: **T4-pMV7**

Catalog number: 158

Provided: 1 vial of transformed bacteria.

Cloning vector: pMV7.

Host: HB101.

Cloning site: *Eco*RI.

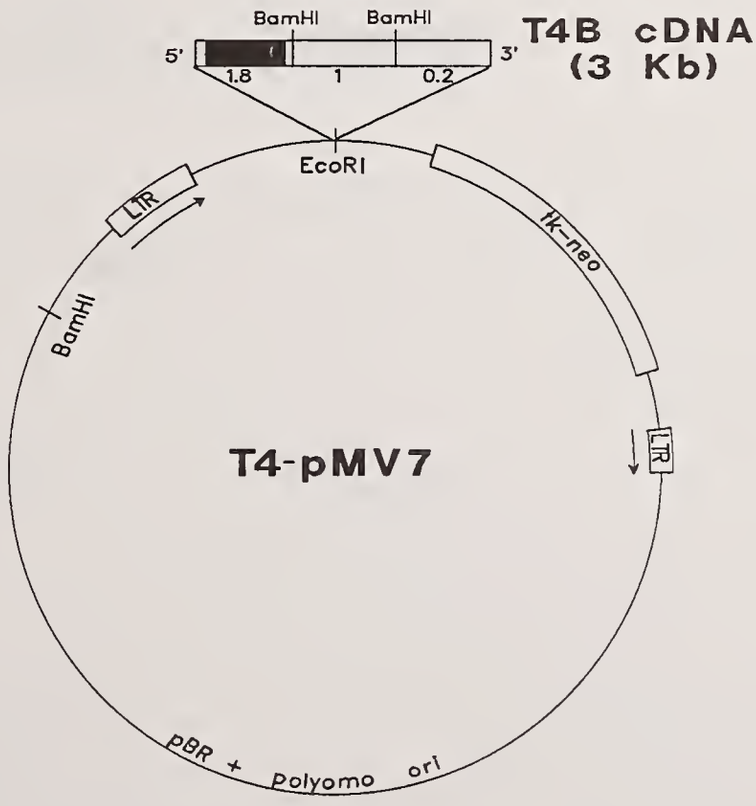
Description of clone: T4-pMV7 is a recombinant retroviral expression vector expressing the human CD4 receptor in mammalian cells. pMV7 contains two LTR repeats of Moloney murine sarcoma virus spanning a unique *Eco*RI cloning site. pMV7 also contains the bacterial neomycin phosphotransferase gene (*neo*) fused to the HSV thymidine kinase promoter (*tk*), both located downstream of the cloning site.

Special characteristics: T4-pMV7 contains full length cDNA insert encoding CD4 (3 kb total of which 1.8 kb is the coding sequence). Transfection of T4-pMV7 to a retrovirus helper cell line (AM or φ2) results in the production of replication-defective recombinant retrovirus.

Contributor: Dr. Richard Axel.

References: Maddon, P.J., et al. *Cell* **47**:333, 1986.

NOTE: ***The CD4 gene is in pT4B (catalog #157).***



Reagent: **T8-pMV7**

Catalog number: 159

Provided: 1 vial of transformed bacteria.

Cloning vector: pMV7.

Host: HB101.

Cloning site: *Eco*RI.

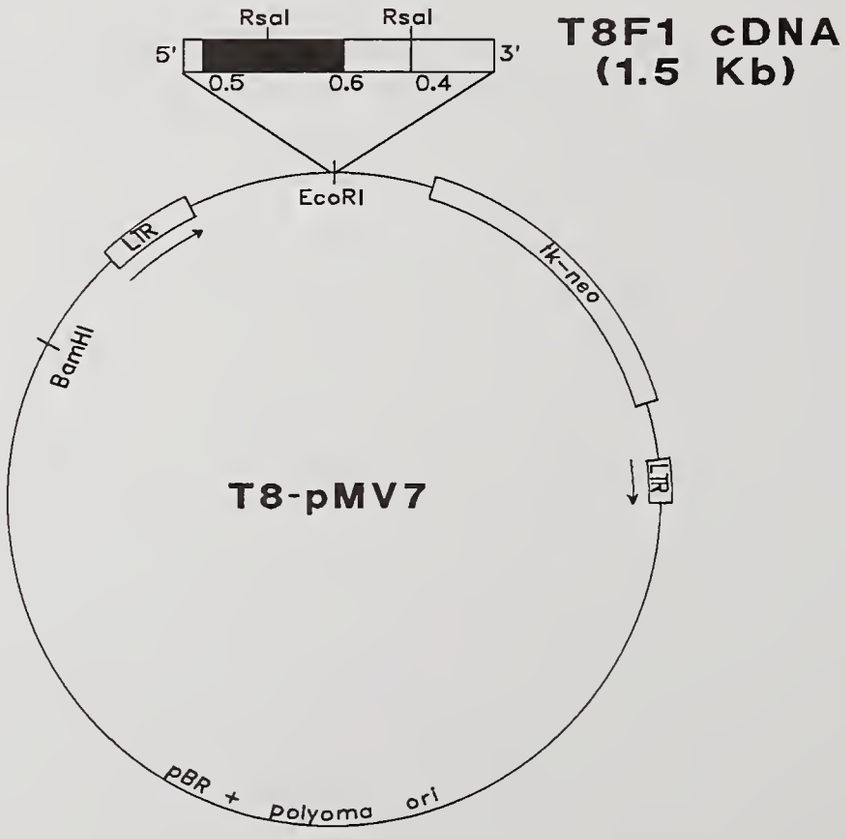
Description of clone: T8-pMV7 is a recombinant retroviral expression vector expressing the human CD8 receptor in mammalian cells. pMV7 contains two LTR repeats of Moloney murine sarcoma virus spanning a unique *Eco*RI cloning site. pMV7 also contains the bacterial neomycin phosphotransferase gene (*neo*) fused to the HSV thymidine kinase promoter (*tk*), both located downstream of the cloning site.

Special characteristics: T8-pMV7 contains full length cDNA insert encoding CD8 (1.5 kb total of which 0.7 kb is the coding sequence). Transfection of T8-pMV7 to a retrovirus helper cell line (AM or φ2) results in the production of replication-defective recombinant retrovirus.

Contributor: Dr. Richard Axel.

References: Maddon, P.J., et al. *Cell* **47**:333, 1986.

NOTE: ***The CD8 gene is in pT8F1 (catalog #179).***



Prepared in Cells/Expressed in Cells

Adenovirus

Reagent: HIV-1CAT-ad

Catalog number: 101

Provided: 1 vial cell-free virus.

Cloning vector: Adenovirus.

Host: 293 cell line.

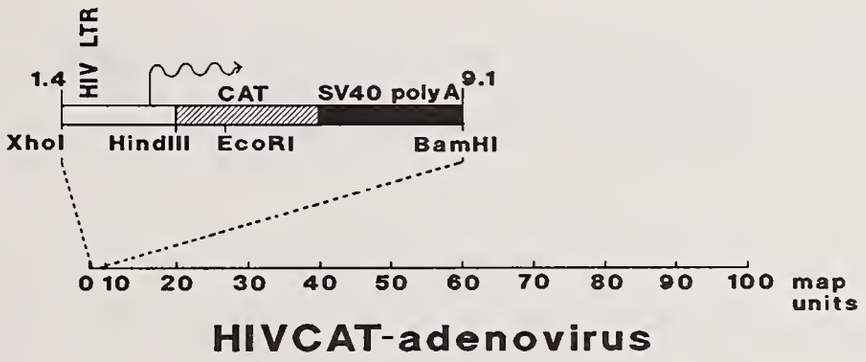
Cloning site: *In vivo* recombinant E1 region.

Description of clone: HIV-1 LTR, fused to CAT, was removed from a bacterial plasmid and incorporated into adenovirus by *in vivo* recombination.

Special characteristics: The recombinant contains sequences of defined HIV-1 LTR regulatory elements including TAR.

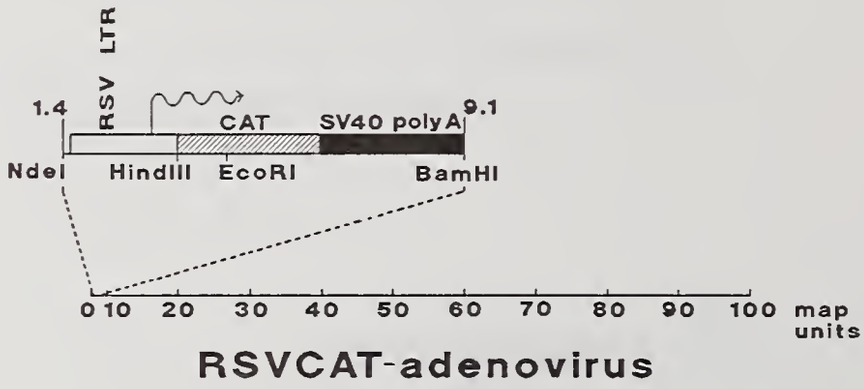
Contributor: Dr. Andrew Rice.

References: Rice, A.P. and Mathews, M.B. *Nature* **332**:551, 1988.



HIVCAT-adenovirus

Reagent:	**RSVCAT-ad**
Catalog number:	102
Provided:	1 vial cell-free virus.
Cloning vector:	Adenovirus.
Host:	293 cell line.
Cloning site:	*In vivo* recombination at the E1 region.
Description of clone:	RSV LTR fused to CAT was removed from a bacterial plasmid and incorporated into adenovirus by *in vivo* recombination.
Special characteristics:	The RSV LTR is insensitive to Tat.
Contributor:	Dr. Andrew Rice.
References:	Rice, A.P. and Mathews, M.B. *Nature* **332**:551, 1988.



RSVCAT-adenovirus

Vaccinia

Reagent: ☣ VV:*gag*

Catalog number: 405

Provided: 1 vial cell-free virus.

Cloning vector: pVV3.

Host: VV:*gag* infects a wide variety of cells. However, expression and processing of *gag* polyprotein may vary significantly among different cells.

Cloning site: *Sac*I.

Cloning strategy: 5.3 kb *Sac*I segment including entire *gag* and *pol* regions from the HXB-C2 molecular clone was cloned into the *Sac*I site of the vaccinia virus recombination vector pVV3 and incorporated into vaccinia virus by homologous recombination.

Description of clone: VV:*gag* expresses high levels of HIV-1 *gag* proteins.

Special characteristics: The *gag* proteins expressed by this recombinant are processed accurately into mature *gag* proteins.

Contributor: Dr. Edgar Engleman.

References: Gowda, S.D., et al. *J. Virol.* **63**:1451, 1989.
Gowda, S.D., Stein, B.S., and Engleman, E.G. *J. Biol. Chem.* **264**:8459, 1989.

Reagent: ☣ vCF21

Catalog number: 360

Provided: 1 vial cell-free virus.

Cloning vector: Vaccinia virus, strain WR.

Host: HeLa and other vertebrate cells.

Cloning site: Thymidine kinase gene.

Description of clone: The clone expresses the reverse transcriptase domain of the *pol* of HIV-1 (clone HXB2). Translation initiation and termination codons were added to the gene which is regulated by the vaccinia virus P7.5 promoter.

Special characteristics: Active reverse transcriptase is expressed. Recombinant vaccinia virus also expresses *E. coli* β-galactosidase.

Contributor: Dr. Charles Flexner and Dr. Bernard Moss.

References: Flexner, C., et al. *Virol.* **166**:339, 1988.

NOTE: ***The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.***

The control vaccinia virus to this clone is available as catalog #353.

Reagent: ☣ **vPE5**

Catalog number: 355

Provided: 1 vial cell-free virus.

Cloning vector: Vaccinia virus, strain WR.

Host: HeLa and other vertebrate cells.

Cloning site: Thymidine kinase gene.

Description of clone: Contains the entire *env* gene of HIV-1 (isolate HTLV-III$_B$, clone BH8) under the control of bacteriophage T7 promoter.

Special characteristics: Expression only occurs when cells are co-infected with a second vaccinia virus expressing bacteriophage T7 RNA polymerase. The gp160 is glycosylated, processed, and inserted into the plasma membrane. Will form syncytia with human CD4 cells.

Contributor: Dr. Patricia Earl and Dr. Bernard Moss.

References: Fuerst, T.R., Earl, P.L., and Moss, *B. Mol. Cell. Biol.* 7:2538, 1987.

NOTE: ***The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.***

The control vaccinia virus to this clone is available as catalog #353.

Reagent: ☣ **vPE6**

Catalog number: 354

Provided: 1 vial cell-free virus.

Cloning vector: Vaccinia virus, strain WR.

Host: HeLa and other vertebrate cells.

Cloning site: Thymidine kinase gene.

Description of clone: Contains the gp120 segment of the *env* gene of HIV-1 (isolate HTLV-III$_B$, clone BH8) under the control of the bacteriophage T7 promoter.

Special characteristics: Expression only occurs when cells are co-infected with a second vaccinia virus expressing bacteriophage T7 RNA polymerase. gp120 is glycosylated, secreted into the medium, and binds to CD4.

Contributor: Dr. Patricia Earl and Dr. Bernard Moss.

References: Berger, E.A., Fuerst, T.R., and Moss, B. *Proc. Natl. Acad. Sci. (USA)* **85**:2357, 1988.

NOTE: ***The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.***

The control vaccinia virus to this clone is available as catalog #353.

Reagent: ☣ **vPE8**

Catalog number: 361

Provided: 1 vial cell-free virus.

Cloning vector: Vaccinia virus, strain WR.

Host: HeLa and other vertebrate cells.

Cloning site: Thymidine kinase gene.

Description of clone: This clone expresses gp120 derived from the *env* gene of HIV-1 (isolate HTLV-III$_B$, clone BH8). The *env* gene was truncated to eliminate the gp41 coding segment and a stop codon was introduced. The truncated *env* gene is regulated by the vaccinia virus P7.5 promoter.

Special characteristics: The gp120 polypeptide is glycosylated and secreted from infected cells. Binding to CD4 has been demonstrated. Recombinant vaccinia virus also expresses *E. coli* β-galactosidase.

Contributor: Dr. Patricia Earl and Dr. Bernard Moss.

References: Manuscript in preparation, contact source.

NOTE: *The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.*

The control vaccinia virus to this clone is available as catalog #353.

Reagent: ☣ **vPE16**

Catalog number: 362

Provided: 1 vial cell-free virus.

Cloning vector: Vaccinia virus, strain WR.

Host: HeLa and other vertebrate cells.

Cloning site: Thymidine kinase gene.

Description of clone: The entire *env* gene of HIV-1 (isolate HTLV-III$_B$, clone BH8) is expressed. It is regulated by the vaccinia virus P7.5 promoter.

Special characteristics: The *env* gene has been modified to eliminate cryptic vaccinia virus early transcriptional stop signals without altering coding sequences. Expresses gp160 which is glycosylated, processed into gp120 and gp41, and inserted into the plasma membrane. Cells infected with vPE16 will form syncytia with human CD4$^+$ cells. The recombinant vaccinia virus also expresses *E. coli* β-galactosidase.

Contributor: Dr. Patricia Earl and Dr. Bernard Moss.

References: Manuscript in preparation, contact source.

NOTE: *The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.*

The control vaccinia virus to this clone is available as catalog #353.

Reagent: ☣ vSC8

Catalog number: 357

Provided: 1 vial cell-free virus.

Cloning vector: Vaccinia virus, strain WR.

Host: HeLa and other vertebrate cells.

Cloning site: Thymidine kinase gene.

Description of clone: The *E. coli lacZ* (β-galactosidase) gene, under the control of the vaccinia virus P11 promoter, is expressed.

Special characteristics: May be used as a control for recombinant vaccinia viruses that express HIV-1 genes and β-galactosidase.

Contributor: Dr. Sekhar Chakrabarti and Dr. Bernard Moss.

References: Chakrabarti, S., Brechling, K., and Moss, B. *Mol. Cell. Biol.* **5**:3403, 1985.

NOTE: ***The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.***

The control vaccinia virus to this clone is available as catalog #353.

Reagent: ☣ vSC40

Catalog number: 359

Provided: 1 vial cell-free virus.

Cloning vector: Vaccinia virus, strain WR.

Host: HeLa and other vertebrate cells.

Cloning site: Thymidine kinase gene.

Description of clone: Contains the *gag-pol* gene of HIV-1 (isolate HTLV-III$_B$, clone BH10). The entire gene is regulated by the vaccinia virus P7.5 promoter.

Special characteristics: Principal products are p55 and p41 *gag* proteins. p55 is myristilated; little or no reverse transcriptase is expressed. Recombinant vaccinia virus also expresses *E. coli* β-galactosidase.

Contributor: Dr. Sekhar Chakrabarti and Dr. Bernard Moss.

References: Flexner, C., et al. *Virol.* **166**:339, 1988.

NOTE: ***The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.***

The control vaccinia virus to this clone is available as catalog #353.

Reagent: ☣ **vTF7-3**

Catalog number: 356

Provided: 1 vial cell-free virus.

Cloning vector: Vaccinia virus, strain WR.

Host: HeLa and other vertebrate cells.

Cloning site: Thymidine kinase gene.

Description of clone: The entire T7 RNA polymerase gene is expressed under the control of the vaccinia P7.5 promoter.

Special characteristics: Active T7 RNA polymerase is made. Used in conjunction with vaccinia viruses that have genes under control of bacteriophage T7 promoters.

Contributor: Dr. Tom Fuerst and Dr. Bernard Moss.

References: Fuerst, et al. *Proc. Natl. Acad. Sci. (USA)* **83**:8122, 1986.

NOTE: ***The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.***

The control vaccinia virus to this clone is available as catalog #353.

Reagent: ☣ **vVK1**

Catalog number: 358

Provided: 1 vial cell-free virus.

Cloning vector: Vaccinia virus, strain WR.

Host: HeLa and other vertebrate cells.

Cloning site: Thymidine kinase gene.

Description of clone: Contains the *gag-pol* gene of HIV-1 (clone HXB2). The entire gene is regulated by the vaccinia virus P7.5 promoter.

Special characteristics: Expression and processing of *gag-pol* and formation of active reverse transcriptase occurs. Recombinant vaccinia virus also expresses *E. coli* β-galactosidase.

Contributor: Dr. Velissarios Karacostas and Dr. Bernard Moss.

References: Karacostas, V., et al. *Proc. Natl. Acad. Sci. (USA)* **86**:8964, 1989.

NOTE: ***The government has filed a patent application on this research material. Corporate requests should be directed to the National Technical Information Service (NTIS), Federal Licensing Office, (703) 487-4732.***

The control vaccinia virus to this clone is available as catalog #353.

PURIFIED PROTEINS

Human Immunodeficiency Virus 1

Reagent: **gp120 from HIV-1_{SF2}**

Catalog number: 386

Provided: 1 ml (50 μg).

Molecular weight: Approximately 120,000.

Glycosylation: Yes.

Degree of purity: 90%.

Production system: Genetically engineered Chinese hamster ovary cells (CHO).

Special characteristics: The purified material binds to CD4, the HIV-1 receptor. The N-terminus has been sequenced and shown to be glu 31 lys 32 with a perfect match to the previously published sequence for viral gp120. The C-terminus should be identical to the viral-derived molecule based on sequences engineered into the expression vector used for its production.

Contributor: Dr. Nancy Haigwood.

References:
Haigwood, N.L., et al. Manuscript submitted.
Levy, J.A., et al. *Science* **225**:840, 1984.
Sanchez-Pescador, R., et al. *Science* **227**:484, 1985.

NOTE: ***Limited to 1 aliquot per laboratory.***

Reagent: **Non-glycosylated gp120 from HIV-2_{SF2}**

Catalog number: 388

Provided: 100 μg (1.3 mg/ml).

Molecular weight: 55,000.

Glycosylation: No.

Degree of purity: >90%.

Production system: Genetically engineered yeast.

Special characteristics: This molecule corresponds to the entire amino acid sequence of HIV-1_{SF2} (originally ARV-2, Levy et al. *Science* **225**:840) gp120 produced as a non-glycosylated molecule in yeast. It elicits neutralizing antibodies in animals effective against HIV-1_{SF2} but not other divergent HIV-1 isolates. This material must be warmed at 45°C for 2-3 hours prior to aliquoting to ensure complete solubilization. If the protein is to be tested *in vitro* with live cells, it must be diluted at least 1/100 or the cells will lyse.

Contributor: Dr. Kathelyn Steimer.

References:

Barr, P.J. Personal communication.
Haigwood, N.L., et al. Manuscript submitted.
Steimer, K.S., et al. *Vaccines 88: New Chemical and Genetic Approaches to Vaccination: Prevention of AIDS and Other Viral, Bacterial, and Parasitic Diseases*, ed. Ginsberg, H., et al., 347-355. Cold Spring Harbor, NY: Cold Spring Harbor Laboratories, 1988.

NOTE: ***Limited to 1 aliquot per laboratory.***

Reagent: **gp120**

Catalog number: 420

Provided: 50 μg.

Glycosylation: Yes.

Degree of purity: 90% (10% gp70, 50) as determined by SDS-PAGE.

Production System: Baculovirus expression sytem.

Activity: Equivalent to reference HIV-1 derived gp120 as determined by soluble CD4 based ELISA.

Contributor: Purchased by the Repository from American Bio-Technologies, Inc.

References: Personal communication.

NOTE: ***Limited to one aliquot per laboratory.***

Reagent: **gp120, HIV-1 Recombinant**

Catalog number: 534

Provided: 0.5 ml (100 μg).

Glycosylation: Yes.

Degree of purity: >95% as determined by densitometry analysis of a Coomassie blue stained non-reduced SDS-PAGE. Less than 15% cleaved to 70kD and 50kD polypeptides as seen by densitometric scan of a Coomassie blue stained reduced sample on SDS-PAGE.

Production System: Genetically engineered Chinese hamster ovary cells (CHO).

Activity: Immunoactive in ELISA using DuPont monoclonal anti-gp120 (NEA 9284) to enable capture and Aalto sheep anti-gp120 polyclonal (D7324) for detection.

Special characteristics: Co-migrates with a rHIV-1 gp120 standard on SDS-PAGE. Detected by Western blots probed with anti-gp120 rabbit polyclonal serum. This batch of HIV-1 gp120 has been purified by immunoaffinity chromatography and subsequently dialysed with 20 mM HEPES, pH 7.0. The dialysate was then concentrated and filtered through a 0.2 μm filter prior to rapid freezing and storage at -70°C.

Contributor: Purchased by the Repository from Celltech, Inc.

References: Personal communication.

NOTE: ***Limited to one aliquot per laboratory.***

Reagent: **HIV-1 Protease**

Catalog number: 457

Provided: 100 μg.

Molecular weight: 10,000.

Glycosylation: None.

Degree of purity: 50%.

Production system: Host cells are *E. coli* K12 cells. The expression vector is pET3A.

Special characteristics: HIV protease was isolated as inclusion bodies. It was stabilized with 8 M urea. Active enzyme can be regenerated by dilution or dialysis. A protocol for regeneration of active enzyme will be included with the shipment.

Contributor: Dr. Y.S. Edmond Cheng.

References: Personal communication.

NOTE: ***Limited to one aliquot per laboratory.***

Reagent: **Reverse Transcriptase (recombinant)**

Catalog number: 419

Provided: 10 μl (2 μg).

Production system: *E. coli.*

Origin: HIV-1 (clone BH-10).

Degree of purity: 90%.

Activity: 2.8×10^5 pmole/min/mg.

Contributor: Dr. Christine Debouck.

References: Mizrahi, V., et al. *Arch. Biochem. Biophys.* **273**:347, 1989.

NOTE: ***Limited to one aliquot per laboratory.***

Reagent: **Reverse Transcriptase (recombinant)**

Catalog number: 454

Provided: 200 μl (93.2 μg).

Molecular weight: 46.5% 66 kD, 21.1% 53 kD, 32.3% 55 kD as determined by gel scanning.

Production system: *E. coli.*

Origin: HIV-1 (clone BH-10).

Activity: 2,000 units/mg protein. One unit is defined as the amount of enzyme which will catalyze the incorporation of 1 nmole of TMP into DNA in 10 min. at 37°C.

Special characteristics: Stable at -80°C. Can be frozen and thawed several times without loss of activity. Working solutions that do not contain glycerol can be stored at 4°C for several weeks. Under standard assay conditions, 5 ng of RT in 50 μl reaction will incorporate in 10 minutes at 37°C at a concentration of 10 pmole of dTMP. Standard RT assay mix: 50 mM Tris-HCl, pH 7.9, 60 mM KCl, 7 mM $MgCl_2$, 40 mM DTT, 2 mM GSH, 1.4 μM oligo dT.poly A, 50 μM [α-^{35}S]dTTP (1,000 cpm/pmole) (according to Dr. Christine Debouck of Smith Kline and French).

Contributor: Division of AIDS, NIAID; produced under contract by BioTechnology General.

References: Personal communication.

NOTE: ***Limited to one aliquot per laboratory.***

Reagent: **HIV-1 p25/24 Gag**

Catalog number: 382

Provided: 100 μg (1.43 mg/ml).

Molecular weight: 25,000.

Glycosylation: No.

Degree of purity: >90%.

Production system: Genetically engineered yeast.

Special characteristics: Derived from the *gag* gene of the HIV-1_{SF2} (Levy, et al. *Science* **223**:840) virus isolate.

Contributor: Dr. Kathelyn Steimer.

References: Barr, P.J., et al. *UCLA Symp. Mol. Cell. Biol. New. Ser.* **43**:205, 1987.
Steimer, K.S., et al. *Virol.* **150**:283, 1986.

NOTE: ***Limited to 1 aliquot per laboratory.***

CD4

Reagent: **Recombinant Soluble CD4**

Catalog number: 546

Provided: 100 μg.

Molecular weight: 55,000.

Degree of purity: >95.

Production system: Baculovirus/sf/9 cell expression system.

Activity: Activity assayed by Western immune ELISA, binding to gp120; 10-100 nanograms per well or individual assay recommended.

Special characteristics: The protein was immune-affinity purified and is supplied in 10 mM glycine, pH 7.2. Store at -70°C.

Contributor: Purchased by the Repository from American Bio-Technologies, Inc.

References: Dr. Pat Dimond. Personal communication.

NOTE: ***Limited to one aliquot per laboratory.***

ANTIBODIES, MONOCLONAL

Human Immunodeficiency Virus 1

Reagent: **Monoclonal Antibody to rec.Nef (No. NF2-B2)**

Catalog number: 456

Provided: 200 μl.

Host: Balb/c mice splenocytes x NS1.

Isotype: IgG_1.

Titer: Shows half maximal binding at a dilution of 1:10,000 as evidenced by solid-phase ELISA.

Special characteristics: This antibody was derived from ascitic fluid and obtained from a third subcloning cycle. It reacts with an epitope in the amino terminal part of Nef, as evidenced by a solid phase ELISA utilizing CNBr cleaved polypeptides. Controls used for initial screening and subcloning included nonimmune mouse serum and HAT medium (negative) and hyperimmune mouse serum (positive). Control for ascites titer was nonimmune mouse serum. Control for epitope determination was whole rec.Nef.

Contributor: Division of AIDS, NIAID; produced under contract by BioTechnology General.

References: Personal communication.

Reagent: **Monoclonal Antibody 76C to HIV-1 p25/24 Gag**

Catalog number: 383

Provided: 100 μl (200-1000 μg Ig).

Host: Mouse.

Isotype: IgG_{2b}.

Special characteristics: Antibody 76C reacts with p25/24 Gag in Western blots and radioimmunoprecipitation assays. There it recognizes an epitope visible in both denatured and native p25/24 Gag. It was raised against HIV_{SF2} (Levy, et al. *Science* **225**:840) virus p25/24 Gag but cross-reacts with all other HIV-1 isolates examined to date. It has been successfully used as the capture reagent in a p25 Gag ELISA (see reference).

Contributor: Dr. Kathelyn Steimer.

References: Steimer, et al. *Virol.* **150**:283, 1986.

NOTE: ***Limited to 1 aliquot per laboratory.***

Reagent: **Monoclonal Antibody to HIV-1 (No. 13.10)**

Catalog number: 377

Provided: 330 μl (50 μg).

Host: Human.

Isotype: IgG_1, λ chain.

Special characteristics: Monoclonal antibody produced by cloned B cells from an AIDS patient and P3X63 AgU.1 mouse myeloma cells. Antibody is in HBSS with BSA added. It recognizes gp160 and gp120 by Western blot and binds to whole $HTLV\text{-}III_B$ and fixed or live infected cells.

Contributor: Dr. Evan Hersh and Dr. Yasuhiko Masuho.

References: Lake, D., et al. *Life Sci.* 45:iii, 1989.

NOTE: ***Limited to 1 aliquot per laboratory.***

Corporate requests should be directed in writing to Dr. Hersh at the Department of Medicine, University of Arizona, Tucson, AZ 85724.

Reagent: **Monoclonal Antibody to HIV-1 (No. P5-3)**

Catalog number: 378

Provided: 55 μl (50 μg).

Host: Human.

Isotype: IgG_1, λ chain.

Special characteristics: Monoclonal antibody produced by a heterohybridoma made using B cells from an AIDS patient and P3X63 AgU.1 mouse myeloma cells. Antibody is in HBSS with BSA added. It binds to whole $HTLV\text{-}III_B$ and fixed or live infected cells.

Contributor: Dr. Evan Hersh and Dr. Yasuhiko Masuho.

References: Personal communication.

NOTE: ***Limited to one aliquot per laboratory.***

Corporate requests should be directed in writing to Dr. Hersh at the Department of Medicine, University of Arizona, Tucson, AZ 85724.

Reagent: **Monoclonal Antibody to HIV-1 (No. 86)**

Catalog number: 380

Provided: 100 μl (50 μg).

Host: Human.

Isotype: IgG_1, κ chain.

Special characteristics: Monoclonal antibody produced by a heterohybridoma made using B cells from an AIDS patient and P3X63 AgU.1 mouse myeloma cells. Antibody is in HBSS with BSA added. It recognizes gp160, gp41, and gp120 (weakly) by Western blot and binds to whole $HTLV\text{-}III_B$ and fixed or live infected cells.

Contributor: Dr. Evan Hersh and Dr. Yasuhiko Masuho.

References: Sugano, T., et al. *Biochem. Biophys. Res. Comm.* **155**:1105,1988.

NOTE: *Limited to one aliquot per laboratory.*

Corporate requests should be directed in writing to Dr. Hersh at the Department of Medicine, University of Arizona, Tucson, AZ 85724.

Reagent: **Monoclonal Antibody to HIV-1 (No. V7-8)**

Catalog number: 381

Provided: 50 μl (50 μg).

Host: Human.

Isotype: IgG_3, κ chain.

Special characteristics: Monoclonal antibody produced by a heterohybridoma made using B cells from an AIDS patient and P3X63 AgU.1 mouse myeloma cells. Antibody is in HBSS with BSA added. It recognizes p55 and p24 by Western blot and binds to whole $HTLV\text{-}III_B$ and fixed or live infected cells.

Contributor: Dr. Evan Hersh and Dr. Yasuhiko Masuho.

References: Personal communication.

NOTE: *Limited to one aliquot per laboratory.*

Corporate requests should be directed in writing to Dr. Hersh at the Department of Medicine, University of Arizona, Tucson, AZ 85724.

Reagent: **Monoclonal Antibody to HIV-1 (No. N2-4)**

Catalog number: 528

Provided: 100 μg.

Host: HIV^+ human B-cell/P3X63 AgU.1 mouse myeloma cell hybridomas.

Isotype: IgG_1, κ chain.

Special characteristics: With the Bio-Rad Western blotting kit, N2-4 reacts with gp160, gp41, and weakly with gp120.

Contributor: Dr. Evan Hersh.

References: Personal communication.

NOTE: *Limited to one aliquot per laboratory.*

Reagent: **Monoclonal Antibody to HIV-1 (No. R9-2)**

Catalog number: 529

Provided: 100 μg.

Host: HIV^+ human B cell/P3X63 AgU.1 mouse myeloma cell hybridomas.

Isotype: IgG_1, λ chain.

Special characteristics: With the Bio-Rad Western blotting kit, R9-2 reacts with gp160 and gp120.

Contributor: Dr. Evan Hersh.

References: Personal communication.

NOTE: *Limited to one aliquot per laboratory.*

Reagent: **Monoclonal Antibody to HIV-1 p24 Core Protein**

Catalog number: 389

Provided: 100 μl (100 μg).

Host: Mouse.

Isotype: IgG_1, κ chain.

Contributor: Dr. Paul Yoshihara.

References: Personal communication.

NOTE: *Available only as a single shipment of 100 μg of bioreactor supernatant per laboratory.*

Reagent: **Monoclonal Antibody to HIV-1 p24 (No. 71-31)**

Catalog number: 530

Provided: 1 ml (12.4 μg).

Host: Human.

Isotype: IgG, λ chain.

Titer: For ELISA, a 1:1000 dilution is recommended. For ADCC, a 1:10 dilution is recommended.

Special characteristics: This antibody is anti-p24, and reacts with HIV lysate in ELISA. It reacts with the p55 core precursor as well as p24 and several intermediates as determined by Western blot. The antibody stains the cytoplasm of HIV-1 infected cell lines.

Contributor: Dr. Susan Zolla-Pazner.

References: Gorny, M.K., et al. *Proc. Natl. Acad. Sci. (USA)* **86**:1624, 1989.

NOTE: *Corporate requests should be directed in writing to Dr. Susan Zolla-Pazner at the Veterans Administration Medical Center, 408 First Avenue, New York, NY 10010.*

Reagent: **Monoclonal Antibody to HIV-1 gp41 (No. 50-69)**

Catalog number: 531

Provided: 1 ml (18 μg).

Host: Human.

Isotype: IgG_2, κ chain.

Titer: For ELISA, a dilution of 1:1000 is recommended. For ADCC assays, a dilution of 1:10 is recommended.

Special characteristics: This antibody is anti-HIV gp41 and reacts by ELISA with a DuPont gp41 peptide of amino acids 560-642. It also reacts with Labsystems test using a gp41 peptide 599-613. This represents the most immunodominant portion of gp41. The antibody is also reactive on Western blot with trimers and tetramers of gp41 (less so with the monomer). The antibody stains the membrane of HIV-1 infected cell lines.

Contributor: Dr. Susan Zolla-Pazner.

References: Gorny, M.K., et al. *Proc. Natl. Acad. Sci. (USA)* **86**:1624, 1989.
Till, M.A., et al. *Proc. Natl. Acad. Sci. (USA)* **86**:1987, 1989.
Pinter, A., et al. *J. Virol.* **63**:2674, 1989.

NOTE: *Corporate requests should be directed in writing to Dr. Susan Zolla-Pazner at the Veterans Administration Medical Center, 408 First Avenue, New York, NY 10010.*

HTLV-I

Reagent:	**Monoclonal Antibody, 0.5 Alpha**
Catalog number:	309
Provided:	1 ml.
Host:	Human.
Isotype:	IgG_1, κ chain.
Special characteristics:	Monoclonal antibody is produced by an Epstein-Barr virus transformed B cell clone (0.5 Alpha). It binds to the cell membrane of T cells infected with HTLV-I and lyses them in the presence of complement. The antibody does not react with HTLV-I negative T cells. In electroblot assays, the monoclonal antibody detects a 46 kD glycoprotein in disrupted HTLV-I virions and a 34 kD product following digestion of the viral protein with endoglycosidase F.
Contributor:	Dr. Samuel Broder.
References:	Matsushita, S., et al. *Proc. Natl. Acad. Sci. (USA)* **83**:2672, 1986.

ANTIBODIES, POLYCLONAL

Human Immunodeficiency Virus 1

Reagent: **Antiserum to rec.Nef**

Catalog number: 455

Provided: 200 μl undiluted antiserum.

Host: Rabbit.

Titer: Shows half maximal binding at a 1:5000 dilution of the antiserum as demonstrated by a solid-phase immunoassay utilizing iodinated protein A.

Special characteristics: The control for titer determination was nonimmune rabbit serum.

Contributor: Division of AIDS, NIAID; produced under contract by BioTechnology General.

References: Personal communication.

Reagent: **Antiserum to HIV-1 p25/24 Gag**

Catalog number: 384

Provided: 200 μl undiluted antiserum.

Host: Rabbit.

Titer: ELISA: >1:100,000.

Special characteristics: This polyclonal serum reacts in Western blots, ELISAs, and RIP assays with viral p25/24 Gag and can also be used to capture or detect native p25/24 Gag in detergent lysates of HIV-1 virus. It cross-reacts with all HIV-1 isolates tested to date. It was generated by immunizing rabbits with purified p25/24 Gag produced in *E. coli*.

Contributor: Dr. Kathelyn Steimer.

References: Steimer, K.S., et al. *Virol.* **150**:283, 1986.

NOTE: ***Limited to 1 aliquot per laboratory.***

Reagent: **Antiserum to HIV-1_{SF2} gp120**

Catalog number: 385

Provided: 200 μl undiluted antiserum.

Host: Goat.

Titer: >1:100,000 by ELISA.

Special characteristics: This antibody was raised against glycosylated gp120. It cross-reacts with gp120 from multiple HIV-1 isolates in ELISA and Western blot assays. It also neutralizes HIV-1_{SF2}, the virus strain from which the immunogen originated but not HIV-1_{BRU}, the other isolate which has been examined.

Contributor: Dr. Nancy Haigwood.

References:
Haigwood, N.L., et al. Manuscript submitted.
Levy, J.A., et al. *Science* **225**:840, 1984.
Sanchez-Pescador, R., et al. *Science* **227**:484, 1985.

NOTE: *Limited to 1 aliquot per laboratory.*

Reagent: **Antiserum to Non-glycosylated HIV-1_{SF2} gp120**

Catalog number: 387

Provided: 250 μl undiluted antiserum.

Host: Goat.

Titer: >250,000.

Special characteristics: This antibody was raised against non-glycosylated gp120. It cross-reacts with gp120 from multiple HIV-1 isolates in Western blot assays of virus. Also cross reacts with non-glycosylated gp120 analogs from other isolates in ELISA assays. The serum neutralizes HIV-1_{SF2}, the virus strain from which the DNA for its production was derived, but not any of the other HIV-1 isolates that have been examined to date.

Contributor: Dr. Kathelyn Steimer.

References:
Barr, P.T. Personal communication.
Haigwood, N.L., et al. Manuscript submitted.
Steimer, K.S., et al. 1988. *Vaccines '88: New Chemical and Genetic Approaches to Vaccination: Prevention of AIDS and Other Viral, Bacterial, and Parasitic Diseases*, ed. Ginsberg, H., et al., 347-355. New York: Cold Spring Harbor Laboratories.

NOTE: *Limited to 1 aliquot per laboratory.*

Reagent: **Antiserum to Nef, N-terminal End**

Catalog number: 464

Provided: 200 μl undiluted antiserum.

Host: Rabbit.

Titer: Not determined. The contributor uses the antiserum at a dilution of 1:250-1:500 for immunoprecipitation and at a dilution of 1:1000-1:2000 for immunofluorescence.

Special characteristics: This antibody was raised against a synthetic peptide spanning amino acids 2-28 inclusive of the Nef protein of the HIV-1_{HXB-3} strain conjugated to KLH. The exact sequence of the peptide is CGGKWSKSSVVGWPAV-RERMRRAEPAAD. This antibody is very good for immunofluorescence, and also works for immunoprecipitation.

Contributor: Dr. Bryan Cullen.

References: Hammes, S.R., et al. *Proc. Natl. Acad. Sci. (USA)* **86**, In press.

Reagent: **Antiserum to Nef, C-terminal End**

Catalog number: 465

Provided: 1 ml undiluted antiserum.

Host: Rabbit.

Titer: Not determined. The contributor uses the antiserum at a dilution of 1:250-1:500 for immunoprecipitation and at a dilution of 1:1000-1:2000 for immunofluorescence.

Special characteristics: This antibody was raised against a synthetic peptide spanning amino acids 171-184 inclusive of the Nef protein of the HIV-1_{HXB-3} strain conjugated to KLH. The exact sequence of the peptide is CHGMDDPEREV-LEWRFDSR. This antibody is excellent for immunoprecipitation, but poor for immunofluorescence.

Contributor: Dr. Bryan Cullen.

References: Hammes, S.R., et al. *Proc. Natl. Acad. Sci. (USA)* **86**, In press.

Reagent: **Antiserum to PB1$_B$**

Catalog number: 36

Provided: 200 μl undiluted antiserum.

Host: Goat.

Special characteristics: Antiserum is specific for the PB1 domain of gp160 from HTLV-III$_B$ (amino acid residues 295-474). Protein is derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References:
Matsushita, S., et. al. *J. Virol.* **62**:2107, 1988.
Putney, S.D., et. al. *Science* **234**:1392, 1986.
Rusche, J.R., et. al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to PB1 Sub 7**

Catalog number: 38

Provided: 200 μl undiluted antiserum.

Host: Goat.

Special characteristics: Antiserum is specific for PB1 subclone 7 domain of gp160 from HTLV-III$_B$ (amino acid residues 350-455). Protein is derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References:
Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Putney, S.D., et al. *Science* **234**:1392, 1986.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to PB1 Sub 2**

Catalog number: 40

Provided: 200 μl undiluted antiserum.

Host: Goat.

Special characteristics: Antiserum is specific for PB1 subclone 2 domain of gp160 from HTLV-III$_B$ (amino acid residues 295-404). Protein is derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References:
Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Putney, S.D., et al. *Science* **234**:1392, 1986.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to $PB1_{MN}$ and $PB1_{RF}$**

Catalog number: 41

Provided: 200 μl undiluted antiserum.

Host: Goat.

Special characteristics: Antiserum was obtained by co-inoculation of PB1 domains from HTLV-III_{MN} and HTLV-III_{RF} (amino acid residues 295-474). Protein is derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References:
Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Putney, S.D., et al. *Science* **234**:1392, 1986.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to $PB1_{SC}$**

Catalog number: 43

Provided: 200 μl undiluted antiserum.

Host: Goat.

Special characteristics: Antiserum is specific for PB1 domain of gp160 from HTLV-III_{SC} (amino acid residues 295-474). Protein is derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References:
Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Putney, S.D., et al. *Science* **234**:1392, 1986.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to $PB1_{RF}$**

Catalog number: 45

Provided: 200 μl undiluted antiserum.

Host: Goat.

Special characteristics: Antiserum is specific for the PB1 domain of gp160 from HTLV-III_{RF} (amino acid residues 295-474). Protein is derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References:
Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Putney, S.D., et al. *Science* **234**:1392, 1986.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to PB1 Sub 2 - CN1**

Catalog number: 46

Provided: 200 μl undiluted antiserum.

Host: Goat.

Special characteristics: Antiserum is specific for PB1 subclone 2-CN1 domain of gp160 from HTLV-III$_B$ (amino acid residues 295-333). Protein is derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References: Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Putney, S.D., et al. *Science* **234**:1392, 1986.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to PB1$_{MN}$**

Catalog number: 47

Provided: 200 μl undiluted antiserum.

Host: Goat.

Special characteristics: Antiserum is specific for the PB1 domain of gp160 from HTLV-III$_{MN}$ (amino acid residues 295-474). Protein is derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References: Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Putney, S.D., et al. *Science* **234**:1392, 1986.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to gp160$_B$ and gp160$_{RF}$ (HT3-HT7)**

Catalog number: 51

Provided: 200 μl undiluted antiserum.

Host: Goat.

Special characteristics: Antiserum was obtained by co-inoculation of gp160 from HTLV-III$_B$ and HTLV-III$_{RF}$ and reacts with both proteins, which are derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References: Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to PB1 Sub 6**

Catalog number: 57

Provided: 200 μl undiluted antiserum.

Host: Goat.

Special characteristics: Antiserum is specific for PB1 subclone 6 domain of gp160 from HTLV-III_B (amino acid residues 350-474). Protein is derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References: Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Putney, S.D., et al. *Science* **234**:1392, 1986.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to $gp160_B$ (HT3)**

Catalog number: 188

Provided: 200 μl undiluted antiserum.

Host: Goat.

Titer: 1:3000-15,000 obtained by endpoint dilution with ELISA against preimmune serum from same animal.

Special characteristics: Antiserum is specific for the entire sequence of HTLV-III_B gp160 (BH10) derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References: Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci.(USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to $gp160_{RF}$ (HT7)**

Catalog number: 189

Provided: 200 μl undiluted antiserum.

Host: Goat.

Titer: 1:3000-15,000 obtained by endpoint dilution with ELISA against preimune serum from same animal.

Special characteristics: Antiserum is specific for the entire sequence of HTLV-III_{RF} gp160 derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References: Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to $gp160_B/gp160_{RF}$ Hybrid (HT6)**

Catalog number: 190

Provided: 200 μl undiluted antiserum.

Host: Goat.

Titer: 1:3000-15,000 obtained by endpoint dilution with ELISA against preimmune serum from same animal.

Special characteristics: Antiserum is specific for gp160 of $HTLV\text{-}III_B$ containing a substituted PB1 domain from $HTLV\text{-}III_{RF}$ (amino acid residues 295-474). Protein is derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References: Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to $gp160_B$ and $gp160_{RF}$**

Catalog number: 191

Provided: 200 μl undiluted antiserum.

Host: Goat.

Titer: 1:3000-15,000 obtained by endpoint dilution with ELISA against preimmune serum from same animal.

Special characteristics: Antiserum was obtained by co-inoculation of gp160 from $HTLV\text{-}III_B$ and $HTLV\text{-}III_{RF}$ and reacts with both proteins, which are derived from baculovirus. Descriptive map appears after Cat. No. 56.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References: Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***

Reagent: **Antiserum to PB1$_{WMJ2}$**

Catalog number: 56

Provided: 200 μl undiluted antiserum.

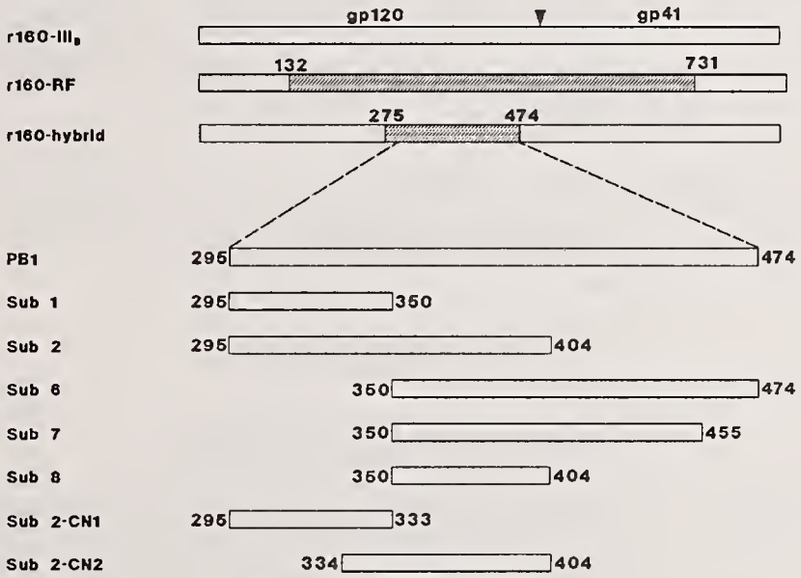
Host: Goat.

Special characteristics: Antiserum is specific for PB1 domain of gp160 of HTLV-III$_{WMJ2}$ (amino acid residues 295-474). Protein is derived from baculovirus. Descriptive map appears below.

Contributor: Division of AIDS, NIAID; produced under contract by Repligen.

References:
Matsushita, S., et al. *J. Virol.* **62**:2107, 1988.
Putney, S.D., et al. *Science* **234**:1392, 1986.
Rusche, J.R., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:6924, 1987.

NOTE: ***The molecular clone used to express this recombinant antigen was distributed by Dr. Robert Gallo of the Laboratory of Tumor Cell Biology, Division of Cancer Etiology, National Cancer Institute.***



Reagent: **Antiserum to HIV-1 Protease C-terminal Peptide**

Catalog number: 226

Provided: 200 μl undiluted antiserum.

Host: Rabbit.

Titer: 1:1000 by ELISA and Western blot.

Special characteristics: Use at 1:1000 dilution. Antiserum is directed against peptide but reacts strongly with BSA. For Western blot, block with 1% nonfat dry milk with 0.1% Tween-20 in PBS. Serum should be stored at -90°C.

Contributor: Dr. Bruce Korant.

References: Personal communication.

Reagent: **Antiserum to gp120$_{MN}$**

Catalog number: 363

Provided: 0.5 ml undiluted antiserum.

Host: Goat.

Titer: Will be on data sheet sent with sample.

Special characteristics: The synthetic peptide SP10, containing a type specific determinant against the 3rd variable domain (V3) of gp120 from HTLV-III$_{MN}$ (amino acid sequence 303-321) was coupled to a 16 amino acid T cell epitope (T1) of HTLV-III$_B$ (amino acid sequence 428-443) and used to raise goat antibodies against HIV gp120. The resultant serum neutralizes HTLV-III$_{MN}$ isolates in a type specific manner.

Contributor: Dr. T.J. Palker, Dr. T.J. Matthews, Dr. A. Langlois, Dr. D.P. Bolognesi, and Dr. B.F. Haynes.

References:
Palker, T.J., et al. *J. Immunol.* **142**:3612, 1989.
Palker, T.J., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:2479, 1987.
Palker, T.J., et al. *Proc. Natl. Acad. Sci. (USA)* **85**:1932, 1988.

NOTE: *Limited to one aliquot per laboratory.*

Reagent: **Antiserum to gp120$_B$**

Catalog number: 364

Provided: 0.5 ml undiluted antiserum.

Host: Goat.

Titer: Will be on data sheet sent with sample.

Special characteristics: The synthetic peptide SP10, containing a type specific determinant against the 3rd variable domain (V3) of gp120 from HTLV-III$_B$ (amino acid sequence 303-321) was coupled to tetanus toxoid and used to raise goat antibodies against HIV-1 gp120. The resultant serum neutralizes HTLV-III$_B$ isolates in type specific manner.

Contributor: Dr. T.J. Palker, Dr. T.J. Matthews, Dr. A.J. Langlois, Dr. D.P. Bolognesi, and Dr. B.F. Haynes.

References:
Palker, T.J., et al. *J. Immunol.* **142**:3612, 1989..
Palker, T.J., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:2479, 1987.
Palker, T.J., et al. *Proc. Natl. Acad. Sci. (USA)* **85**:1932, 1988.

NOTE: *Limited to one aliquot per laboratory.*

A negative control to this antiserum is offered as catalog #365.

Reagent: **Antiserum to gp120$_{RF}$**

Catalog number: 366

Provided: 0.5 ml undiluted antiserum.

Host: Goat.

Titer: Will be on data sheet sent with sample.

Special characteristics: The synthetic peptide SP10, containing a type specific determinant against the 3rd variable domain (V3) of gp120 from HTLV-III$_{RF}$ (amino acid sequence 303-321) was coupled to tetanus toxoid and used to raise goat antibodies against HIV-1 gp120. The resultant serum neutralizes HTLV-III$_{RF}$ isolates in a type specific manner.

Contributor: Dr. T.J. Palker, Dr. T.J. Matthews, Dr. A.J. Langlois, Dr. D.P. Bolognesi, and Dr. B.F. Haynes.

References:
Palker, T.J., et al. *J. Immunol.* **142**:3612, 1989.
Palker, T.J., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:2479, 1987.
Palker, T.J., et al. *Proc. Natl. Acad. Sci. (USA)* **85**:1932, 1988.

NOTE: ***Limited to one aliquot per laboratory.***

A negative control to this antiserum is offered as catalog #365.

Reagent: ☣ **Pedigreed Panel of Plasma from HIV-1 Infected Individuals**

Catalog number: 543

Provided: 35 1 ml specimens:

26 plasma from HIV-1 infected people
4 duplicates
3 dilutions of one specimen
2 plasma pools and HIV-1 Ig prepared therefrom

Host: Human.

Titer: Neutralizing, p24, and ADCC titers will be provided on the data sheet.

Special characteristics: The pedigreed panel has been tested by several research groups. The study was directed by Dr. Luiz Barbosa of the National Heart, Lung, and Blood Institute.

Contributor: National Heart, Lung, and Blood Institute.

References:
Proc. Natl. Acad. Sci. (USA) **85**:6944, 1988.

NOTE: ***To obtain this panel, please write directly to Dr. Luiz Barbosa and enclose a protocol detailing the proposed use of the panel. Dr. Barbosa can be reached at:***

National Heart, Lung, and Blood Institute
Federal Building, Room 504
7550 Wisconsin Avenue
Bethesda, MD 20892

Reagent: **Antiserum to HIV-1 p17**

Catalog number: 286

Provided: 1 vial lyophilized protein.

Host: Sheep.

Titer: ELISA: 1:1700; Neutralization: <1:8; Immunofluorescence: 1:320; Western blot: p17/p55: $10^{-4}/10^{-4}$.

Special characteristics: Lyophilized polyclonal serum specific for HIV-1 p17 as well as its parent and degradation products. The materials in the vials are free of bacterial contamination and contain NO preservatives. When reconstituted in 1.5 ml sterile distilled water, the sera are neat with respect to unprocessed sera. SDS-PAGE was used to resolve the p17 protein of HTLV-III$_B$. The pertinent band was excised, ground, and emulsified in Freund's complete adjuvant for primary inoculation and in incomplete Freund's adjuvant for a booster administered 2 months later. Two additional booster doses in RIBI were given at the 3rd and 4th months followed by a final boost in Freund's incomplete at 15 months. The animals were maintained by the Ungulate Unit at the NIH Animal Facility and plasmapheresed during the 16th and 17th months.

Contributor: Dr. Michael Phelan.

References: Personal communication.

Reagent: **Antiserum to HIV-1 p24**

Catalog number: 287

Provided: 1 vial lyophilized protein.

Host: Sheep.

Titer: ELISA: 1:600; Neutralization: <1:8; Immunofluorescence: 1:80; Western blot: p24/p55: $10^{-3}/10^{-3}$.

Special characteristics: Lyophilized polyclonal serum specific for HIV-1 p24 as well as its parent and degradation products. The materials in the vials are free of bacterial contamination and contain NO preservatives. When reconstituted in 1.5 ml sterile distilled water, the sera are neat with respect to unprocessed sera. SDS-PAGE was used to resolve the p24 protein of HTLV-III$_B$. The pertinent band was excised, ground, and emulsified in Freund's complete adjuvant for primary inoculation and in incomplete Freund's adjuvant for a booster administered 2 months later. Two additional booster doses in RIBI were given at the 3rd and 4th months followed by a final boost in Freund's incomplete at 15 months. The animals were maintained by the Ungulate Unit at the NIH Animal Facility and plasmapheresed during the 16th and 17th months.

Contributor: Dr. Michael Phelan.

References: Personal communication.

Reagent: **Antiserum to HIV-1 gp120**

Catalog number: 288

Provided: 1 vial lyophilized protein.

Host: Sheep.

Titer: ELISA: 1:25,000; Neutralization: 1:256; Immunofluorescence: 1:640; Western blot: gp120/160: $10^{-4}/10^{-5}$.

Special characteristics: Lyophilized polyclonal serum specific for HIV-1 gp120 as well as its parent and degradation products. The materials in the vials are free of bacterial contamination and contain NO preservatives. When reconstituted in 1.5 ml sterile distilled water, the sera are neat with respect to unprocessed sera. Recombinant antigen was emulsified in Freund's complete adjuvant for primary inoculation and in incomplete Freund's adjuvant for the booster doses given at 1 and 4 months. The animal was plasmapheresed during the 5th month by the Ungulate Unit, at the NIH Animal Facility.

Contributor: Dr. Michael Phelan.

References: Personal communication.

Reagent:: **Human Antibody to HIV-1 RT**

Catalog number: 187

Provided: 200 μl.

Host: Human.

Isotype: Various polyclonal IgG.

Titer: Concentration is 1 mg/ml.

Special characteristics: IgG was isolated from serum of individuals exposed to HIV-1, using salt precipitation and ion-exchange. Protein is in PBS with no azide, sterile and frozen.

Contributor: Dr. Jeffrey Laurence.

References: Laurence, J., et al. *Science* **235**:1501, 1987.

NOTE: ***Limited to one aliquot per laboratory.***

Reagent: **Human HIV-1 Immune Globulin**

Catalog number: 192

Provided: 1 vial lyophilized protein.

Host: Human.

Isotype: 97-98% various polyclonal IgG; 2-3% various other polyclonal Ig. Ig is 86% 7S monomer.

Titer: 1:500-3200 obtained by HIV-1 neutralizing tests. Titer is 1:640,000 by ELISA.

Special characteristics: This preparation was used to test the protective effect of high-titer neutralizing antibody in chimpanzees challenged with HIV-1. Gamma globulin was prepared from pooled plasma of healthy HIV-1 seropositive donors. A four-step virus inactivation and removal procedure resulted in a preparation which was non-infective and without adverse effect at 10cc/kilo in chimpanzees. This material is lyophilized and should be resuspended in 1 ml distilled water before use. The preparation was originally suspended in 0.01 M $NaPO_4$, pH 6.2-7.0, 0.003 M sodium citrate, and 4.5% glucose. This preparation can be used as a standard.

Contributor: Dr. Alfred Prince and the National Heart, Lung, and Blood Institute.

References: Prince, A.M., et al. *Proc. Natl. Acad. Sci. (USA)* **85**:6944, 1988.

Human Immunodeficiency Virus 2

Reagent: **Antiserum to HIV-2 Tat**

Catalog number: 466

Provided: 1 ml undiluted antiserum.

Host: Rabbit.

Titer: Not determined. The contributor uses the antiserum at a dilution of 1:250-1:500 for immunoprecipitation and at a dilution of 1:1000-1:2000 for immunofluorescence.

Special characteristics: This antibody was raised against a synthetic peptide spanning amino acids 76-99 inclusive of the Tat protein of the HIV-2_{ROD} strain conjugated to keyhole limpet hemocyanin (KLH). The exact sequence of the peptide is CYERKGRRRRTPKKTKTHPSPTPDK. The antibody is very good for both immunoprecipitation and immunofluorescence. It does not react with HIV-1 or SIV Tat.

Contributor: Dr. Bryan Cullen.

References: Malim, M.H., et al. *Proc. Natl. Acad. Sci. (USA)* **86**:8222, 1989.

Reagent: **Antiserum to HIV-2Z Peptide**

Catalog number: 257

Provided: 200 μl undiluted antiserum.

Host: Goat.

Titer: Greater than 1:100,000 by radioimmunoassay against peptide.

Special characteristics: Antiserum was raised by hyperimmunization with a synthetic peptide (CMSGFLFHSQPVINKKPRQ) from gp120 of HIV-2Z coupled to tetanus toxoid.

Contributor: Dr. Thomas Palker and Dr. Bart Haynes.

References: Personal communication.

NOTE: *Limited to one aliquot per laboratory.*

Pre-immune serum is available as catalog #258.

Reagent: ☣ **HIV-2 Serum Reference Panel**

Catalog number: 409

Provided: 1 set.

Host: Human.

Titer: Additional information will be on a data sheet included with each shipment.

Special characteristics: Information on content will be included with the shipment.

Contributor: Dr. Saladin Osmanov.

References: Personal communication.

HTLV-I

Reagent: **Antiserum to HTLV-I Tax**

Catalog number: 467

Provided: 1 ml undiluted antiserum.

Host: Rabbit.

Titer: Not determined. The contributor uses the antiserum at a 1:250-1:500 dilution for immunoprecipitation and at a dilution of 1:1000-1:2000 for immunofluorescence.

Special characteristics: This antibody was raised against a synthetic peptide spanning the last 32 amino acids inclusive of the Tax protein conjugated to keyhole limpet hemocyanin(KLH). The exact sequence of the peptide is NEKEADENDHEPQISPGGLEPPSEKHFRETEV. This antibody is excellent for both immunofluorescence and immunoprecipitation.

Contributor: Dr. Bryan Cullen.

References: Hanly, S.M., et al. *Genes and Dev.* 3:1534, 1989.

Simian Immunodeficiency Virus

Reagent: **Antiserum to SIV_{agm}**

Catalog number: 241

Provided: 200 μl undiluted antiserum.

Host: Monkey (pigtail).

Titer: 1:2560 by ELISA.

Special characteristics: Antiserum is from SIV infected African green monkey. Antibodies are reactive against intact virus particles and viral proteins.

Contributor: Dr. Maneth Gravell.

References: Personal communication.

Reagent: Antiserum to SIV_{smm}

Catalog number: 242

Provided: 200 μl undiluted antiserum.

Host: Monkey (rhesus).

Titer: 1:10,240 by ELISA.

Special characteristics: Antiserum is from SIV infected sooty mangaby monkey. Antibodies are reactive against intact virions and viral proteins.

Contributor: Dr. Maneth Gravell.

References: Personal communication.

Other

Reagent: Antiserum to Tetanus Toxoid-MBS

Catalog number: 365

Provided: 0.5 ml undiluted antiserum.

Host: Goat.

Special characteristics: Goats were immunized with tetanus toxoid treated with m-maleimido-benzoyl-N-hydroxysuccinimide ester (MBS).

Contributor: Dr. T.J. Palker, Dr. T.J. Matthews, Dr. A.J. Langlois, Dr. D.P. Bolognesi, and Dr. B.F. Haynes.

References:
Palker, T.J., et al. *J. Immunol.* **142**:3612, 1989.
Palker, T.J., et al. *Proc. Natl. Acad. Sci. (USA)* **84**:2479, 1987.
Palker, T.J., et al. *Proc. Natl. Acad. Sci. (USA)* **85**:1932, 1988.

NOTE: ***Limited to one aliquot per laboratory.***

The resultant serum serves as a negative control to antisera raised against $SP10III_B$ (catalog #364) and $SP10III_{RF}$ (catalog #366).

Reagent: **Antiserum to Human CD4**

Catalog number: 314

Provided: 1 vial

Host: Sheep.

Titer: ELISA: 1:10,000.

Special characteristics: Lyophilized polyclonal serum specific for human CD4 as well as its parent and degradation products. The materials in the vials are free of bacterial contamination and contain NO preservatives. When reconstituted in 1.5 ml sterile distilled water, the sera are neat with respect to unprocessed sera. Recombinant antigen was emulsified in Freund's complete adjuvant for primary inoculation and in incomplete Freund's adjuvant for the booster doses given at 1 and 4 months. The animal was plasmapheresed during the 5th month by the Ungulate Unit, at the NIH Animal Facility.

Contributor: Dr. Michael Phelan.

References: Personal communication.

Reagent: **Normal Sheep Serum**

Catalog number: 315

Provided: 1 vial

Host: Sheep.

Special characteristics: Lyophilized polyclonal serum. The materials in the vials are free of bacterial contamination and contain NO preservatives. When reconstituted in 1.5 ml sterile distilled water, the sera are neat with respect to unprocessed sera.

Contributor: Dr. Michael Phelan.

References: Personal communication.

BIOLOGICAL RESPONSE MODIFIERS

Reagent: **Human rIL-2**

Catalog number: 136

Provided: 1×10^6 units.

Specific activity: 1×10^6 units/ml (referenced to BRMP standard).

Shipping conditions: Dry ice.

Special characteristics: No endotoxin detected by LAL assay. Lyophilized preparation contains 25 mg human serum albumin and 5 mg mannitol. Dilute with 1 ml sterile normal saline solution at physiological pH. Aliquot and store at -20°C.

Contributor: Dr. Maurice Gately.

References: Lahm, H.-W. and Stein, S. *J. Chrom.* **326**:357, 1985.

Reagent: **GCT Media**

Catalog number: 147

Provided: 50 ml

Shipping conditions: Dry ice.

Storage conditions: -20°C.

Special characteristics: For maintenance and growth of primary human monocytes. Conditioned media is generated in 10% fetal bovine serum. Generally used at 10% final concentration. When human monocytes/macrophages (M/M) were cultured in Iscove's Modified Dulbecco's Medium with 10% GCT conditioned medium and 10% human serum, , and co-cultured with PBMs from HIV-1 infected individuals, efficiency of HIV-1 isolation was higher than in the absence of GCT conditioned medium. GCT conditioned medium also increased the replication of HIV-1 when passaged from infected M/M to fresh M/M.

Contributor: Division of AIDS, NIAID, with GCT cells provided by Dr. James K. Brennan.

References: Di Persio, J.F., et al. *Blood* **51**:507, 1978.

NOTE: *Serum-free GCT Media is available as catalog #412.*

Limited to 50 ml per 2 months. Special requests will be considered depending on availability and should be addressed to:

Dr. Nava Sarver
Section Chief
Targetted Drug Discovery Section
Developmental Therapeutics Branch
Division of AIDS , NIAID
6003 Executive Blvd.
Rockville, MD 20892
301-496-8197.

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Appendix A: Safety Guidelines–HIV

CENTERS FOR DISEASE CONTROL

April 1, 1988 / Vol. 37 / No. S-4

MMWR

Supplement

MORBIDITY AND MORTALITY WEEKLY REPORT

1988
Agent Summary Statement for Human Immunodeficiency Virus

and

Report on Laboratory-Acquired Infection with Human Immunodeficiency Virus

U.S. Department of Health and Human Services
Public Health Service
Centers for Disease Control
Center for Infectious Diseases
Hospital Infections Program
AIDS Program
Atlanta, Georgia

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Agent Summary Statement for Human Immunodeficiency Viruses (HIVs) Including HTLV-III, LAV, HIV-1, and HIV-2*

INTRODUCTION

In 1984, the Centers for Disease Control (CDC) and the National Institutes of Health (NIH), in consultation with experts from academic institutions, industry, and government, published the book *Biosafety in Microbiological and Biomedical Laboratories* ("*Guidelines*")† (*1*).

These *Guidelines* are based on combinations of standard and special practices, equipment, and facilities recommended for use in working with infectious agents in various laboratory settings. The recommendations are advisory; they provide a general code for operating microbiologic and biomedical laboratories.

One section of the *Guidelines* is devoted to standard and special microbiologic practices, safety equipment, and facilities for biosafety levels (BSL) 1 through 4. Another section contains specific agent summary statements, each consisting of a brief description of laboratory-associated infections, the nature of laboratory hazards, and recommended precautions for working with the causative agent. The authors realized that the discovery of the availability of information about these agents would necessitate updating the agent summary. Such a statement for human immunodeficiency virus (HIV) (called HTLV-III/LAV when the *Guidelines* were published) was published in *MMWR* in 1986 (*2*). The HIV agent summary statement printed in this *Supplement* updates the 1986 statement.

Attached to the updated HIV agent summary statement are the essential elements for BSL 2 and 3 laboratories, reproduced from the *Guidelines* (*1*) (see Addendum 1, p. 6). BSL 2 and 3 laboratory descriptions are included because they are recommended for laboratory personnel working with HIV, depending on the concentration or quantity of virus or the type of laboratory procedures used.

*The information and recommendations contained in this document were developed and compiled by the Division of Safety, National Institute of Allergy and Infectious Diseases, the National Cancer Institute, and the Clinical Center of the National Institutes of Health; Food and Drug Administration; and the following CDC units: AIDS Program, Hospital Infections Program, Office of the Director, Center for Infectious Diseases; the Training and Laboratory Program Office; and the Office of Biosafety, Office of the Centers Director; Representatives of the following organizations also collaborated in the effort: the American Academy of Microbiology, the American Biological Safety Association, the American Society for Microbiology, the American Society for Clinical Pathology, the Association of State and Territorial Public Health Laboratory Directors, the College of American Pathologists, the Pharmaceutical Manufacturers Association, and the Walter Reed Army Institute for Research.

†Available from Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402, Stock #01702300167-1; or from National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, Stock #PB84-206879.

The HIV agent summary statement does not specifically address safety measures for collecting and handling clinical specimens. Nonetheless, it has been recommended that blood and body-fluid precautions consistently be used for **ALL** specimens from **ALL** patients. This approach, referred to as "universal blood and body-fluid precautions" or "universal precautions," eliminates the need to identify all patients infected with HIV (or other bloodborne pathogens) (*3*). This subject is also covered in other publications (*3-8*).

Laboratory directors, supervisors, and others are asked to attach a copy of this revised "1988 Agent Summary Statement for Human Immunodeficiency Virus" to each copy of the *Guidelines* and to all copies of their laboratory biosafety manual; they should review the recommended precautions with laboratory personnel, provide appropriate training in practices and operation of facilities, and ensure that all personnel demonstrate proficiency **BEFORE** being allowed to work with HIV. The laboratory director (or the designated laboratory supervisor) is responsible for biosafety in the laboratory and must establish and implement practices, facilities, equipment, training, and work assignments as appropriate (*9*).

HIV AGENT SUMMARY STATEMENT

Agent: HIVs Including HTLV-III, LAV, HIV-1, and HIV-2

In the period 1984-1986, several health-care workers (HCWs) who had no recognized risk behavior for acquired immunodeficiency syndrome (AIDS) were reported to have HIV infection (*10-15*). Only one of these HCWs was identified as a laboratory worker. These and other reports assessed the risk of work-related HIV infection for all HCWs as being very low (*3,6,10-12,14-18*).

In 1985, anecdotal reports were received indicating that workers in two different HIV-reagent-production laboratories had been exposed to droplets and splashed liquid from a vessel containing concentrated virus. One of several workers had been cut by glass from a broken carboy that contained HIV-infected cells and medium. None of the persons exposed in these episodes had developed antibody to HIV or had clinical signs of infection 18 and 20 months, respectively, after the reported exposure.

In 1987, CDC received reports that three HCWs had HIV infection; none of the infections were associated with needlesticks or cuts. Two of these HCWs were clinical laboratory workers (*11*). One was a phlebotomist whose face and mouth were splattered with a patient's blood when the rubber stopper was suddenly expelled from a blood-collection tube. The second was a medical technologist who inadvertently spilled blood on her arms and forearms while using an apheresis apparatus to process blood from an HIV-seropositive patient.

In September 1987, a production-laboratory worker was reported to have HIV infection (*18*). This person worked with large concentrations of HIV in a BSL 3 facility. HIV was isolated from the worker's blood; the isolate was genetically indistinguishable from the strain of virus being cultivated in the laboratory. No risk factors were identified, and the worker recalled no specific incident that might have led to infection. However, there were instances of leakage of virus-positive culture fluid from equipment and contamination of the work area and centrifuge rotors. The report

concluded that the most plausible source of exposure was contact of the worker's gloved hand with virus-culture supernatant, followed by inapparent exposure to skin.

In October 1987, a second person who worked in another HIV production facility was reported to have HIV infection (*18*). This laboratory was a well-equipped BSL 3 facility, and BSL 3 practices were being followed. This worker reported having sustained a puncture wound to a finger while cleaning equipment used to concentrate HIV.

Laboratory Hazards

HIV has been isolated from blood, semen, saliva, tears, urine, cerebrospinal fluid, amniotic fluid, breast milk, cervical secretions, and tissue of infected persons and experimentally infected nonhuman primates. In the laboratory, virus should be presumed to be present in all HIV cultures, in all materials derived from HIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.

In the laboratory, the skin (especially when scratches, cuts, abrasions, dermatitis, or other lesions are present) and mucous membranes of the eye, nose, mouth, and possibly the respiratory tract should be considered as potential pathways for entry of virus. Needles, sharp instruments, broken glass, and other sharp objects must be carefully handled and properly discarded. Care must be taken to avoid spilling and splashing infected cell-culture liquid and other virus-containing materials.

Recommended Precautions

1. BSL 2 standards and special practices, containment equipment, and facilities, as described in the CDC-NIH publication *Biosafety in Microbiological and Biomedical Laboratories* (*Guidelines*), are recommended for activities involving all clinical specimens, body fluids, and tissues from humans or from infected or inoculated laboratory animals. These are the same standards and practices recommended for handling all clinical specimens. For example, and for emphasis:
   a. Use of syringes, needles, and other sharp instruments should be avoided if possible. Used needles and disposable cutting instruments should be discarded into a puncture-resistant container with a lid. Needles should not be re-sheathed, bent, broken, removed from disposable syringes, or otherwise manipulated by hand.
   b. Protective gloves should be worn by all personnel engaged in activities that may involve direct contact of skin with potentially infectious specimens, cultures, or tissues. Gloves should be carefully removed and changed when they are visibly contaminated. Personnel who have dermatitis or other lesions on the hands and who may have indirect contact with potentially infectious material should also wear protective gloves. Hand washing with soap and water immediately after infectious materials are handled and after work is completed—**EVEN WHEN GLOVES HAVE BEEN WORN** as described above—should be a routine practice.
   c. Generation of aerosols, droplets, splashes, and spills should be avoided. A biological safety cabinet should be used for all procedures that might generate aerosols or droplets and for all infected cell-culture manipulations. The *Guidelines* (pp. 11-13) contain additional precautions for operating at BSL 2.

2. Activities such as producing research-laboratory-scale amounts of HIV, manipulating concentrated virus preparations, and conducting procedures that may produce aerosols or droplets should be performed in a BSL 2 facility with the additional practices and containment equipment recommended for BSL 3 (*19*) (*Guidelines,* pp. 14-17).
3. Activities involving industrial-scale, large-volume production or high concentration and manipulation of concentrated HIV should be conducted in a BSL 3 facility using BSL 3 practices and equipment (*19*).
4. BSL 2 practices, containment equipment, and facilities for animals are recommended for activities involving nonhuman primates and any animals experimentally infected or inoculated with HIV. Because laboratory animals may bite, throw feces or urine, or expectorate at humans, animal-care personnel, investigators, technical staff, and other persons who enter the animal rooms should wear coats, protective gloves, coveralls or uniforms, and—as appropriate—face shields or surgical masks and eye shields to protect the skin and mucous membranes of the eyes, nose, and mouth.
5. All laboratory glassware, disposable material, and waste material suspected or known to contain HIV should be decontaminated, preferably in an autoclave, before it is washed, discarded, etc. An alternate method of disposing of solid wastes is incineration.
6. Laboratory workers should wear laboratory coats, gowns, or uniforms when working with HIV or with material known or suspected to contain HIV. There is no evidence that laboratory clothing poses a risk for HIV transmission; however, clothing that becomes contaminated with HIV preparations should be decontaminated before being laundered or discarded. Laboratory personnel must remove laboratory clothing before going to nonlaboratory areas.
7. Work surfaces should be decontaminated with an appropriate chemical germicide after procedures are completed, when surfaces are overtly contaminated, and at the end of each work day. Many commercially available chemical disinfectants (*5,20-23*) can be used for decontaminating laboratory work surfaces, for some laboratory instruments, for spot cleaning of contaminated laboratory clothing, and for spills of infectious materials. Prompt decontamination of spills should be standard practice.
8. Universal precautions are recommended for handling all human blood specimens for hematologic, microbiologic, chemical, serologic testing; these are the same precautions for preventing transmission of all bloodborne infections including hepatitis B (*17,21,24,25*). It is not certain how effective 56 C-60 C heat is in destroying HIV in serum (*22,23,26*), but heating small volumes of serum for 30 minutes at 56 C before serologic testing reduces residual infectivity to below detectable levels. Such treatment causes some false-positive results in HIV enzyme immunoassays (*27-30*) and may also affect some biochemical assays performed on serum (*27,31,32*).
9. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL 2 (*Guidelines,* pp. 11-13). Addendum 2 (p. 16) to this report is a statement issued by CDC on the use of all human control and reagent serum specimens shipped to other laboratories. The Food and Drug Administration requires that manufacturers of human serum reagents use a similarly worded statement.

10. Medical surveillance programs should be in place in all laboratories that test specimens, do research, or produce reagents involving HIV. The nature and scope of a surveillance program will vary according to institutional policy and applicable local, state, and Federal regulations (*9*).
11. If a laboratory worker has a parenteral or mucous-membrane exposure to blood, body fluid, or viral-culture material, the source material should be identified and, if possible, tested for the presence of virus. If the source material is positive for HIV antibody, virus, or antigen, or is not available for examination, the worker should be counseled regarding the risk of infection and should be evaluated clinically and serologically for evidence of HIV infection. The worker should be advised to report on and to seek medical evaluation of any acute febrile illness that occurs within 12 weeks after the exposure (*3*). Such an illness—particularly one characterized by fever, rash, or lymphadenopathy—may indicate recent HIV infection. If seronegative, the worker should be retested 6 weeks after the exposure and periodically thereafter (e.g., at 12 weeks and 6 months after exposure). During this follow-up period—especially during the first 6-12 weeks after exposure, when most infected persons are expected to show serologic evidence of infection—exposed workers should be counseled to follow Public Health Service recommendations for preventing transmission of HIV (*3,14,25,33*). It is recommended that all institutions establish written policies regarding the management of laboratory exposure to HIV; such policies should deal with confidentiality, counseling, and other related issues.
12. Other primary and opportunistic pathogenic agents may be present in the body fluids and tissues of persons infected with HIV. Laboratory workers should follow accepted biosafety practices to ensure maximum protection against inadvertent laboratory exposure to agents that may also be present in clinical specimens (*34-36*).
13. Unless otherwise dictated by institutional policy, the laboratory director (or designated laboratory supervisor) is responsible for carrying out the biosafety program in the laboratory. In this regard, the laboratory director or designated supervisor should establish the biosafety level for each component of the work to be done and should ensure that facilities and equipment are adequate and in good working order, that appropriate initial and periodic training is provided to the laboratory staff, and that recommended practices and procedures are strictly followed (*9*).
14. Attention is directed to a "Joint Advisory Notice" of the Departments of Labor and Health and Human Services (*9*) that describes the responsibility of employers to provide "safe and healthful working conditions" to protect employees against occupational infection with HIV. The notice defines three exposure categories of generic job-related tasks and describes the protective measures required for employees involved in each exposure category. These measures are: administrative measures, training and education programs for employees, engineering controls, work practices, medical and health-care practices, and record-keeping. The recommendations in this report are consistent with the "Joint Advisory Notice"; managers/directors of all biomedical laboratories are urged to read this notice.

ADDENDUM 1

LABORATORY BIOSAFETY LEVEL CRITERIA

Biosafety Level 2

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents that represent a moderate hazard for personnel and the environment. It differs in that a) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists, b) access to the laboratory is limited when work is being conducted, and c) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2:

A. Standard microbiological practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress.
2. Work surfaces are decontaminated at least once a day and after any spill of viable material.
3. All Infectious liquid or solid waste is decontaminated before being disposed of.
4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food must be stored in cabinets or refrigerators designed and used for this purpose only. Food storage cabinets or refrigerators should be located outside the work area.
6. Persons are to wash their hands when they leave the laboratory after handling infectious material or animals.
7. All procedures are performed carefully to minimize the creation of aerosols.

B. Special practices

1. Contaminated materials that are to be decontaminated away from the laboratory are placed in a durable, leakproof container that is closed before being removed from the laboratory.
2. The laboratory director limits access to the laboratory. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
3. The laboratory director establishes policies or procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) enter the laboratory or animal rooms.
4. When an infectious agent being worked with in the laboratory requires special provisions for entry (e.g., vaccination), a hazard warning sign that incorporates the universal biohazard symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the infec-

tious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

5. An insect and rodent control program is in effect.
6. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before leaving the laboratory for nonlaboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.
7. Animals not involved in the work being performed are not permitted in the laboratory.
8. Special care is taken to avoid having skin be contaminated with infectious material; gloves should be worn when handling infected animals and when skin contact with infectious material is unavoidable.
9. All waste from laboratories and animal rooms is appropriately decontaminated before disposal.
10. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) are used for the injection or aspiration of infectious fluid. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
11. Spills and accidents that result in overt exposures to infectious material are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained.
12. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or on the function of the facility.
13. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to follow them.

C. Containment equipment

Biological safety cabinets (Class I or II) or other appropriate personal-protection or physical-containment devices are used when:

1. Procedures with a high potential for creating infectious aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
2. High concentrations or large volumes of infectious agents are used. Some types of materials may be centrifuged in the open laboratory if sealed heads

or centrifuge safety cups are used and if the containers are opened only in a biological safety cabinet.

D. Laboratory facilities

1. The laboratory is designed so that it can be easily cleaned.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.
4. Each laboratory contains a sink for hand washing.
5. If the laboratory has windows that open, they are fitted with fly screens.
6. An autoclave for decontaminating infectious laboratory wastes is available.

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure by inhalation. Laboratory personnel have specific training in handling pathogenic and/or potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal-protection clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for Biosafety Level 3 (e.g., access zone, sealed penetrations, and directional airflow). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories in which facility features satisfy Biosafety Level 2 recommendations if the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

The following standard and special safety practices, equipment, and facilities apply to agents assigned to Biosafety Level 3:

A. Standard microbiological practices

1. Work surfaces are decontaminated at least once a day and after any spill of viable material.
2. All infectious liquid or solid waste is decontaminated before being disposed of.
3. Mechanical pipetting devices are used; mouth pipetting is prohibited.
4. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.
5. Persons wash their hands after handling infectious materials and animals and every time they leave the laboratory.
6. All procedures are performed carefully to minimize the creation of aerosols.

B. Special practices

1. Laboratory doors are kept closed when experiments are in progress.

2. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable, leakproof container that is closed before being removed from the laboratory.
3. The laboratory director controls access to the laboratory and limits access only to persons whose presence is required for program or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
4. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., vaccination), and who comply with all entry and exit procedures enter the laboratory or animal rooms.
5. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign (incorporating the universal biohazard symbol) is posted on all laboratory and animal-room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for vaccinations, respirators, or other personal-protection measures.
6. All activities involving infectious materials are conducted in biological safety cabinets or other physical-containment devices within the containment module. No work is conducted in open vessels on the open bench.
7. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with infectious materials is finished. Plastic-backed paper toweling used on nonperforated work surfaces within biological safety cabinets facilitates clean-up.
8. An insect and rodent control program is in effect.
9. Laboratory clothing that protects street clothing (e.g., solid-front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated before being laundered.
10. Special care is taken to avoid skin contamination with infectious materials; gloves are worn when handling infected animals and when skin contact with infectious materials is unavoidable.
11. Molded surgical masks or respirators are worn in rooms containing infected animals.
12. Animals and plants not related to the work being conducted are not permitted in the laboratory.
13. All waste from laboratories and animal rooms is appropriately decontaminated before being disposed of.
14. Vacuum lines are protected with high-efficiency particulate air (HEPA) filters and liquid disinfectant traps.
15. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle

is integral to the syringe) are used for the injection or aspiration of infectious fluids. Extreme caution is used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused.

16. Spills and accidents that result in overt or potential exposures to infectious material are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided, and written records are maintained.
17. Baseline serum samples for all laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory.
18. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to follow them.

C. Containment equipment

Biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal-protection or physical-containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with infectious materials that pose a threat of aerosol exposure. These include: manipulation of cultures and of clinical or environmental material that may be a source of infectious aerosols; the aerosol challenge of experimental animals; harvesting of tissues or fluids from infected animals and embryonated eggs; and necropsy of infected animals.

D. Laboratory facilities

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high-containment laboratory from access corridors or other laboratories or activities may also be provided by a double-doored clothes-change room (showers may be included), airlock, or other access facility that requires passing through two sets of doors before entering the laboratory.
2. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.
3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
4. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Each laboratory contains a sink for washing hands. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.
6. Windows in the laboratory are closed and sealed.
7. Access doors to the laboratory or containment module are self-closing.

8. An autoclave for decontaminating laboratory wastes is available, preferably within the laboratory.
9. A ducted exhaust-air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow is proper (i.e., into the laboratory). The exhaust air from the laboratory room can be discharged to the outside without being filtered or otherwise treated.
10. The HEPA-filtered exhaust air from Class I or CLass II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every 12 months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble-unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.

VERTEBRATE ANIMAL BIOSAFETY LEVEL CRITERIA

Animal Biosafety Level 2

A. Standard practices

1. Doors to animal rooms open inward, are self-closing, and are kept closed when infected animals are present.
2. Work surfaces are decontaminated after use or spills of viable materials.
3. Eating, drinking, smoking, and storing of food for human use are not permitted in animal rooms.
4. Personnel wash their hands after handling cultures and animals and before leaving the animal room.
5. All procedures are carefully performed to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

B. Special practices

1. Cages are decontaminated, preferably by autoclaving, before being cleaned and washed.
2. Surgical-type masks are worn by all personnel entering animal rooms housing nonhuman primates.
3. Laboratory coats, gowns, or uniforms are worn while in the animal room. This protective clothing is removed before leaving the animal facility.
4. The laboratory or animal-facility director limits access to the animal room only to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when work is in progress. In general, persons who may be at increased risk of acquiring

infection or for whom infection might be unusually hazardous are not allowed in the animal room.

5. The laboratory or animal-facility director establishes policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific requirements (e.g., vaccination) may enter the animal room.
6. When an infectious agent in use in the animal room requires special-entry provisions (e.g., vaccination), a hazard warning sign (incorporating the universal biohazard symbol) is posted on the access door to the animal room. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the animal-facility supervisor or other responsible person(s), and indicates the special requirement(s) for entering the animal room.
7. Special care is taken to avoid contaminating skin with infectious material; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
8. All waste from the animal room is appropriately decontaminated—preferably by autoclaving—before being disposed of. Infected animal carcasses are incinerated after being transported from the animal room in leakproof, covered containers.
9. Hypodermic needles and syringes are used only for the parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused.
10. If floor drains are provided, the drain taps are always filled with water or a suitable disinfectant.
11. When appropriate, considering the agents handled, baseline serum samples from animal-care and other at-risk personnel are collected and stored. Additional serum samples may be collected periodically, depending on the agents handled or the function of the facility.

C. Containment equipment

Biological safety cabinets, other physical-containment devices, and/or personal-protection devices (e.g., respirators, face shields) are used when procedures with a high potential for creating aerosols are conducted. These include necropsy of infected animals, harvesting of infected tissues or fluids from animals or eggs, intranasal inoculation of animals, and manipulation of high concentrations or large volumes of infectious materials.

D. Animal facilities

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping.
2. A sink for washing hands is available in the room that houses infected animals.
3. If the animal facility has windows that open, they are fitted with fly screens.

4. It is recommended, but not required, that the direction of airflow in the animal facility is inward and that exhaust air is discharged to the outside without being recirculated to other rooms.
5. An autoclave that can be used for decontaminating infectious laboratory waste is available in the same building that contains the animal facility.

Animal Biosafety Level 3

A. Standard practices

1. Doors to animal rooms open inward, are self-closing, and are kept closed when work with infected animals is in progress.
2. Work surfaces are decontaminated after use or after spills of viable materials.
3. Eating, drinking, smoking, and storing of food for human use are not permitted in the animal room.
4. Personnel wash their hands after handling cultures or animals and before leaving the laboratory.
5. All procedures are carefully performed to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

B. Special practices

1. Cages are autoclaved before bedding is removed and before they are cleaned and washed.
2. Surgical-type masks or other respiratory protection devices (e.g., respirators) are worn by personnel entering rooms that house animals infected with agents assigned to Biosafety Level 3.
3. Wrap-around or solid-front gowns or uniforms are worn by personnel entering the animal room. Front-button laboratory coats are unsuitable. Protective gowns must remain in the animal room and must be decontaminated before being laundered.
4. The laboratory director or other responsible person limits access to the animal room only to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when infected animals are present. In general, persons who may be at increased risk of acquiring infection or for whom infection might be unusually hazardous are not allowed in the animal room.
5. The laboratory director or other responsible person establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., vaccination) may enter the animal room.
6. Hazard warning signs (incorporating the universal biohazard warning symbol) are posted on access doors to animal rooms containing animals infected with agents assigned to Biosafety Level 3 are present. The hazard warning sign should identify the agent(s) in use, list the name and telephone number of the animal room supervisor or other responsible person(s), and indicate any special conditions of entry into the animal room (e.g., the need for vaccinations or respirators).
7. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room waste before being disposed of or reused.

8. All wastes from the animal room are autoclaved before being disposed of. All animal carcasses are incinerated. Dead animals are transported from the animal room to the incinerator in leakproof, covered containers.
9. Hypodermic needles and syringes are used only for gavage or parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) are used. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused. When possible, cannulas should be used instead of sharp needles (e.g., gavage).
10. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.
11. If vacuum lines are provided, they are protected with HEPA filters and liquid disinfectant traps.
12. Boots, shoe covers, or other protective footwear and disinfectant footbaths are available and used when indicated.

C. Containment equipment

1. Personal-protection clothing and equipment and/or other physical-containment devices are used for all procedures and manipulations of infectious materials or infected animals.
2. The risk of infectious aerosols from infected animals or their bedding can be reduced if animals are housed in partial-containment caging systems, such as open cages placed in ventilated enclosures (e.g., laminar-flow cabinets), solid-wall and -bottom cages covered by filter bonnets, or other equivalent primary containment systems.

D. Animal facilities

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping and is separated from areas that are open to unrestricted personnel traffic within the building. Passage through two sets of doors is the basic requirement for entry into the animal room from access corridors or other contiguous areas. Physical separation of the animal room from access corridors or from other activities may also be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility that requires passage through two sets of doors before entering the animal room.
2. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be cleaned easily. Penetrations in these surfaces are sealed or capable of being sealed to facilitate fumigation or space decontamination.
3. A foot, elbow, or automatically operated sink for hand washing is provided near each animal-room exit door.
4. Windows in the animal room are closed and sealed.
5. Animal room doors are self-closing and are kept closed when infected animals are present.
6. An autoclave for decontaminating wastes is available, preferably within the animal room. Materials to be autoclaved outside the animal room are transported in a covered, leakproof container.

7. An exhaust-air ventilation system is provided. This system creates directional airflow that draws air into the animal room through the entry area. The building exhaust can be used for this purpose if the exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow is proper (i.e., into the animal room). The exhaust air from the animal room that does not pass through biological safety cabinets or other primary containment equipment can be discharged to the outside without being filtered or otherwise treated.
8. The HEPA-filtered exhaust air from Class I or Class II biological safety cabinets or other primary containment devices is discharged directly to the outside or through the building's exhaust system. Exhaust air from these primary containment devices may be recirculated within the animal room if the cabinet is tested and certified at least every 12 months. If the HEPA-filtered exhaust air from Class I or Class II biological safety cabinets is discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble-unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.

ADDENDUM 2

CDC cautionary notice for all human-serum-derived reagents used as controls:

> **WARNING:** Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, this specimen should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories,* 1984, pages 11-13.

If additional statements describing the results of any heat treatment or serologic procedure(s) already performed on the human-serum reagent or control are used in conjunction with the above cautionary notice, these statements should be worded so as not to diminish the impact of the warning that emphasizes the need for universal precautions.

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Occupationally Acquired Human Immunodeficiency Virus Infections in Laboratories Producing Virus Concentrates in Large Quantities:

Conclusions and Recommendations of an Expert Team Convened by the Director of the National Institutes of Health (NIH)

*Reported by Division of Safety, National Institutes of Health**

INTRODUCTION

The recommendations of the expert team are directed to industrial-scale facilities for the production of large quantities of highly concentrated HIV. Their recommendations are similar to and complement those in the preceding "1988 Agent Summary Statement for Human Immunodeficiency Virus," which updates the one published in 1986 (*1*). Laboratory directors and others responsible for the health and safety of laboratory employees working with HIV and HIV-containing material should carefully consider these relevant recommendations and guidelines in developing an appropriate safety program.

COMMITTEE REPORT

Two workers in different laboratories producing large quantities of highly concentrated HIV have been reported to have laboratory-acquired HIV infections (*1*). One worker's infection was presumed to be caused by "undetected skin contact with virus culture supernatant" (*2*). The other worker's infection followed "an injury with a potentially contaminated needle" (*2*). After the first case was identified, the Director of NIH convened a team of experts to investigate the incidents and to visit seven different laboratories that produced large volumes of HIV. After facilities inspections and separate, confidential interviews with the workers, the team prepared a report of their findings. The conclusions and recommendations from that report follow.

*Expert Team: W. Emmett Barkley, PhD, Director, Division of Engineering Services, National Institutes of Health; Robert McKinney, PhD, Director, Division of Safety, National Institutes of Health; John Richardson, DVM, MPH, Biosafety Officer, Emory University; Gerald Schochetman, PhD, Chief, Laboratory Investigations Branch, AIDS Program, Center for Infectious Diseases, Centers for Disease Control; David Henderson, MD, Hospital Epidemiologist, Warren Grant Magnuson Clinical Center, National Institutes of Health.

The most probable cause for the first laboratory-acquired infection was inapparent parenteral exposure. Frequent opportunities for unrecognized direct contact with contaminated materials and surfaces were reported to be present. Gloves of questionable integrity, skin cuts and abrasions, and one episode of a dermatitis-like condition represented portals for possible exposure and routes of infection. The inexperience of the first infected worker in microbiologic procedures and Biosafety Level (BSL) 3 practices, coupled with the reliance on obtaining necessary skills through on-the-job training in a setting in which episodes of contamination may have occurred frequently, suggests that the worker might not have possessed an appropriate level of proficiency when the infection may have occurred.

The most probable cause for the second worker's infection was parenteral inoculation. This worker recalled incurring an injury with a blunt cannula approximately 6 months before the first seropositive sample. Incidents of contamination, such as those reported by the first worker, occurred infrequently in the second worker's laboratory.

Aerosol transmission is considered to be the least likely cause of infection in both cases. Operations in which aerosols may have been generated were carried out in biological safety cabinets to reduce the potential for inhalation exposure. Although some aerosols may have been released during the few reported rotor-seal failures involving the continuous-flow zonal centrifuge, the potential for contact exposure was greater. Aerosol transmission was unlikely because: a) in situations in which overt aerosol exposure has occurred in laboratory and production operations involving HIV, no exposed workers have seroconverted; b) no evidence exists that suggests aerosols may be a natural mode of HIV transmission; c) the probable cause identified above is consistent with documented modes of transmission of bloodborne pathogens in the laboratory.

The occurrence of these two infections emphasizes the finite risk that exists for laboratory workers who handle concentrated preparations of HIV. The conclusions of a National Cancer Institute prospective cohort study (*2*) indicate that this risk is low and may be similar to the risk for infection of health-care workers who have experienced a needlestick injury.

The occupational risk for infection by parenteral exposure is substantially reduced or eliminated by strict adherence to BSL 2 practices. The recommended use of BSL 3 practices for highly concentrated preparations of HIV is appropriate. The review of these two infected laboratory workers does not suggest the need to alter current CDC/NIH biosafety recommendations for HIV or for patient care (*3*), research (*1*), or virus production. There is a need, however, for more proficiency and discipline in laboratory safety practices.

The following recommendations will help assure maintenance of a safe and healthy environment for laboratory and production-facility workers who handle concentrated preparations of HIV:

A. Strictly adhere to standard microbiologic practices and techniques

The most important recommendation is to adhere strictly to standard microbiologic practices and techniques. Persons working with HIV must be aware of potential hazards and must be trained and proficient in practice and techniques necessary for self-protection. Employees must be informed that parenteral exposure is the most serious potential hazard for causing a laboratory-acquired infection. They must be able to recognize how such

exposures occur and how they can be prevented. Although on-the-job training is an acceptable approach for learning techniques and practices, it is imperative that proficiency be obtained **BEFORE** virus is actually handled.

B. Assure that workers are proficient in virus-handling techniques

Selection criteria for employees who will work in production operations or with concentrated preparations of HIV should require experience in the handling of human pathogens or tissue cultures. If an employee has not had such experience, s/he should participate in carefully structured, well-supervised on-the-job training programs.

The director or person in charge of the laboratory or production facility must ensure that personnel are appropriately trained and are proficient in practices and techniques necessary for self-protection. Initial work activities should not include the handling of virus. A progression of work activities should be assigned as techniques are learned and proficiency is developed. Virus should only be introduced into the work activities after the supervisor is confident it can be handled safely.

C. Monitor work practices

Periodically, the biosafety officer or a person with expertise in biosafety should closely observe practices and techniques used in handling HIV. This can be helpful in identifying activities or behavior that may increase the potential for contact with contaminated material or for inapparent parenteral exposures. If deficiencies are noticed, corrective measures should be specified and implemented.

D. Continuously reinforce safe practices

Practices that reduce the potential for direct contact and inapparent parenteral exposure should be continuously reinforced:

- Gloves should always be worn when concentrated preparations of HIV are handled and when contact with a contaminated surface or material may be unavoidable. If a gloved hand accidentally touches a contaminated surface or material, the glove should be removed immediately and the hands washed.
- Work surfaces should be decontaminated at the end of each day and any time contamination is recognized.
- Workers must develop the habit of keeping hands away from the eyes, nose, and mouth in order to avoid potential exposure of mucous membranes. Wearing filter masks and eye goggles or face shields may assist in accomplishing this objective.
- Needles and sharp implements must not be used when HIV is handled unless no acceptable alternative is available. When possible, unbreakable containers should be substituted for glassware, in order to avoid accidental cuts from broken pieces.
- In the absence of advice and consent of an occupational physician or nurse, no worker should handle any virus-containing material when s/he has cuts or skin abrasions on the hands or wrists.

E. Establish a medical surveillance serology program

Each medical facility should have a medical-surveillance serology program. Serum samples should be obtained at least once a year and analyzed for

seroconversion. Results should be reported to individual workers in a timely manner. Counseling services should be available for workers who have positive serologic results. Procedures that maintain strict confidentiality should be adopted.

F. Revalidate integrity of process, transport, and containment equipment

The operational integrity of all equipment used to process, transport, and contain fluids containing HIV should be revalidated at least once a year. The integrity of such equipment should be revalidated after any system failure that releases contaminated fluids into the work environment.

G. Develop production processes that enhance biosafety

Efforts should be made to explore and use production systems and strategies that reduce operational complexity and manual manipulations.

H. Validate efficacy of decontamination methods

Special attention should be given to demonstrating the adequacy of decontamination methods when high organic content, such as cellular debris, is present.

I. Sponsor and conduct biosafety training initiatives

Responsible institutions should orient such programs toward the application of biosafety practices to work involving HIV. Presentation strategies and materials to make the training widely available should be encouraged.

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Appendix B: Safety Guidelines–Vaccinia

Recommended Precautions: The possession and use of variola viruses is restricted to the World Health Organization Collaborating Center for Smallpox and Other Poxvirus Infections located at the Centers for Disease Control, Atlanta, Georgia. Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of poxviruses other than variola that pose an infection hazard to humans. All persons working in or entering laboratory or animal care areas where activities with vaccinia, monkey pox, or cow pox viruses are being conducted should have documented evidence of satisfactory vaccination within the preceeding three years. Activities with vaccinia, cow pox, or monkey pox viruses in quantities or concentrations greater than those present in diagnostic cultures may also be conducted by immunized personnel at Biosafety Level 2, provided that all manipulations of viable materials are conducted in Class I or II biological safety cabinets or other primary containment equipment.

— From **Biosafety in Microbiological and Biomedical Laboratories**, HHS Publication No. (NIH) 88-8395, May 1988, Centers for Disease Control and National Institutes of Health.

INSTRUCTIONS FOR ORDERING REAGENTS

PLEASE READ CAREFULLY (All forms must be typed)

REGISTRATION

All requests for reagents must come through a Laboratory Director (academic institution), or a Director of Research (for-profit institution).

The Annual Repository Registration and Annual Indemnification Forms (Appendix C, pp. 163-165) must be completed and an original copy returned to the Repository. These forms must be signed on pages 164 and 165 by both the registering investigator and an official capable of legally binding the Institution (e.g. president, vice-president, dean, provost, but *not* a department chairman). Those unable to sign the Indemnification Agreement must complete the Non-acceptance of Indemnification Agreement.

After the forms are received and reviewed, an account number will be assigned to qualified investigators. An account number is necessary to order reagents.

ORDERING REAGENTS

Complete both pages of the Reagent Request Form (Appendix D, p. 167) and return it to the Repository. Please remember to include your account number. All requests by graduate students, postdoctoral fellows, research associates, company scientists, etc. must be signed by the registered investigator.

Biohazardous reagents cannot be ordered without a signed Indemnification Agreement. All biohazardous reagents are marked with a ☣ symbol in the body of the catalog and are listed in an index on page 220.

Requests may be sent by FAX to 301-340-9245, but the original signed paperwork must then be mailed.

Appendix C: Annual Repository Registration Form

Please read the instructions on p. 161 before completing this form

Date:
Name:
Title:
Institution:
Department/Subdivision:
Full Address:

For Office Use Only

Acct. # _____

Signature _____

Date _____

Telephone: ( ) _____ FAX Number: ( )_____

Federal Express Number: _____ Curriculum vitae attached ____

Acknowledgment of Source: _____ Initials

I agree to acknowledge in all publications and presentations of studies utilizing reagents supplied by the Repository both the contributors of the reagents and the AIDS Research and Reference Reagent Program. I also agree to provide copies of all articles and abstracts of presentations to the Repository.

Certification of Use: _____ Initials

I certify that all reagents provided by the Repository will be used for research purposes only, in my laboratory only, at this institution only, and only as authorized. The reagents or materials derived from them will not be allowed to come into the possession of any other person except those engaged in research under my direct supervision who accept these restrictions. The reagents will not be used in the manufacture, marketing, or licensing of any commercial product *unless written exceptions* are granted by the donor and the Repository is notified.

Commercial Discoveries: _____ Initials

If discoveries of commercial value result through use of any of the reagents supplied by the Repository, I agree to notify the Repository and to negotiate in good faith to provide fair compensation to the donor(s) of the reagents.

Certification of Compliance with Safety Standards: _____ Initials

I understand that the requested substance(s) may pose health risks to persons handling or in the vicinity of the substance(s), the environment, and the community. In that regard, I certify that I am cognizant of and will employ the appropriate biosafety standards including special practices, equipment and facilities as specified in the Material Data Sheet. I will comply with all applicable Institution and Government health and safety regulations including the Guidelines detailed in "Human T-Lymphotrophic Virus Type III/Lymphoadenopathy-As-

sociated Virus: Agent Summary Statement", MMWR, Volume 37/Number S-4, dated 4/1/88, or the most current revision of the Guidelines. I will directly supervise all users of the reagents and I will assume responsiblity for assuring that those users are cognizant of and comply with safety standards and good laboratory practice.

Assumption of Shipping Costs: _____
Initials

I will assume the costs of shipping reagents from the Repository via Federal Express or other carrier. If I select another carrier, I assume responsibility for confirming that the carrier is willing to ship biohazardous material and will collect shipments from the Repository. You may use my Federal Express Account number or I will make arrangements for prepaid shipments. No shipments will be made until my proposed shipping arrangements are accepted by the Repository.

Reporting Agreement: _____
Initials

I agree to provide the Repository with a 200-300 word description of the planned use of the requested reagents with each request. I agree to provide the Repository with semi-annual summaries of results of research and potential commercial discoveries resulting from the use of the reagents.

Qualified User::

I certify that I am a (specify one) Principal Investigator (Laboratory Director) of a non-profit research laboratory ____ or Director of Research in a commercial organization____.
I have enclosed a curriculum vitae or biographical sketch. My research is supported by [specify type and identification number(s)]:
NIH Research Grant Number _____
Other Federal Funding _____
International/Foreign Support _____
Private Foundation _____
Industry _____
Other _____

_____ _____
Officier of University or Company (Signature) Requestor (Signature)

_____ _____
(Printed name) (Printed name)

_____ _____
(Title) (Title)

Annual Indemnification Form

(February 1, 1990 to January 31, 1991)

Date_____

INDEMNIFICATION AGREEMENT

As a Receiving Party of reagent(s) (the "Substances") from the AIDS Research and Reference Reagent Program, the Recipient Institution, _____ _____, agrees to indemnify and hold harmless the United States, ERC BioServices Corporation, their suppliers, and contributors of reagents, from any claims, costs, damages, or expenses resulting from any injury (including death), damage or loss that may arise from the possession and use of the Substances or any derivative thereof by the Receiving Party. The individual executing this agreement on behalf of the Recipient Institution warrants that the individual has full authority to do so, and to thereby bind the Recipient Institution.

_____
Officer of University or Company (Signature)

_____
(Printed name)

_____
(Title)

_____
(Institution)

_____
Requestor (Signature)

_____
(Printed name)

_____
(Title)

_____
(Institution)

NON-ACCEPTANCE OF INDEMNIFICATION AGREEMENT

The Recipient Institution is unable to comply with the Repository Indemnification Agreement. As a result, the recipient acknowledges that the AIDS Research and Reference Reagent Program will be unable to provide biohazardous materials.

_____
Officer of University or Company (Signature)

_____
(Printed name)

_____
(Title)

_____
(Institution)

_____
Requestor (Signature)

_____
(Printed name)

_____
(Title)

_____
(Institution)

Appendix D: Reagent Request Form

Please read the instructions on p. 161 before completing this form

Account Number:
Date:
From:
Investigator:
Title:
Institution:
Department/Subdivision:
Full Address:

For Office Use Only

_____ Approved
_____ Not Approved

Signature _____
Date _____

Telephone: ( ) _____ FAX number: ( ) _____

Federal Express Number: _____

I request the following reagents from the AIDS Research and Reference Reagent Program:

1. Catalog Number: ____ Item: _____
2. Catalog Number: ____ Item: _____
3. Catalog Number: ____ Item: _____
4. Catalog Number: ____ Item: _____
5. Catalog Number: ____ Item: _____

I agree to adhere to all of the conditions and agreements in my Annual Repository Registration Form. The shipping arrangements for reagents are Federal Express or as described below:

_____

_____

I have provided a 200-300 word description of the planned use of the requested reagents and a list of those users under my direct supervision who will conduct the experiments (see next page).

_____
Requestor (signature)

ABSTRACT OF PROPOSED REAGENT USE (200-300 Words):

PERSONNEL ENGAGED ON PROJECT:

Name	Phone	Position Title and Role in Project

Appendix E: Partial Listing of Organizations Which May Supply AIDS Reagents Directly to Researchers

Researchers may write to the following organizations to discuss donation of small amounts of research reagents or collaborations. Some or all reagents may not be available. In addition, these organizations are under *no obligation* to provide any of these reagents. A detailed research plan may be requested before donation, and the organization may wish to review resulting articles before publication. Please *write* to the listed contact names before attempting to call them; the success of the Repository depends upon the good will of its contributors and the courtesy of the reagent recipients.

Organization/Contact	Reagents
Burroughs Wellcome Dr. Phillip Furman Virology 3030 Cornwallis Road Research Triangle, NC 27709 919-248-4130	• AZT triphosphate
Cetus Corporation Dr. James Meade Research & Development Administration 1400 53rd Street Emeryville, CA 94608 415-420-3287	• Biological response modifiers
Cetus Corporation Dr. Ellen Daniell 1400 53rd Street Emeryville, CA 94608 415-420-3300	• Reagents and technology for polymerase chain reaction
Chiron Dr. Nancy Haigwood Dr. Kathelyn Steimer 4560 Horton Street Emeryville, CA 10019 415-655-8730	• Monoclonal antibody to HIV-1 gp120 • HIV-1 gp120 • Recombinant HIV-1 antigens expressed in yeast and their antisera
Gilead Sciences Dr. Jeffrey Bird 344 Lakeside Drive Foster City, CA 94464 415-574-3000	• Oligonucleotide analogues and conjugates

Immunex Corporation
Dr. Steven Gillis
51 University Street
Seattle, WA 98101
206-587-0430

- Cytokines
- Colony stimulating factors

New York University
Dr. Susan Zolla-Pazner
Veterans Administration
Medical Center
408 First Avenue
New York, NY 10010
212-951-3211

- Human monoclonal antibodies to HIV-1 gp41 and p24

NIH BRMP Repository
Dr. Craig Reynolds
NCI-FCRF
Frederick, MD 21708
301-698-1098

- Biological response modifiers

Roche Diagnostic Systems
Dr. Ravi Pottathil
Building 58, 1st Floor
340 Kingsland Street
Nutley, NJ 07110
201-235-3471

- Recombinant viral proteins
- Biological response modifiers

Smith, Kline and French
Dr. Christine Debouck
Molecular Genetics
Department
709 Swedeland Road
Swedeland, PA 19406
215-270-7636

- pAS expression vectors
- pOTS-*tat*-IIIB
- pSKF-PRO1
- pSKF-PRO2
- pSKF-PRO3
- pSKF-PRO4

Tanox Biosystems
Dr. Michael Fung
Department of Immunology
and Virology
10301 Stella Link
Suite 110
Houston, TX 77025
713-664-2288

- Monoclonal antibodies to HIV-1 gp120

University of Alabama
Dr. Thomas Hodge
Diabetes Research Training
Center Hospital
18th & 7th Avenues South
Room 801
Birmingham, AL 35294
205-934-2516

- HLA typing
- Oligonucleotides

Upjohn
Dr. Gary Tarpley
Cancer & Infectious Diseases
7252-267-4
301 Henrietta Street
Kalamazoo, MI 49001
616-323-4000

- HIV-1 reverse transcriptase
- *tat* expression system

Appendix F: Vendors of AIDS Research Reagents

Following is a partial list of commercial sources for reagents useful in AIDS research. Other companies are encouraged to provide information which will appear in future catalogs.

Organization/Contact	Reagents
Advanced Biotechnologies Inc. 301-470-3220	• Purified viruses • Purified viral proteins • Polyclonal antibody to virus and viral proteins • Growth factors (IL-2)
Aldrich Chemical Dr. Irwin Klundt 800-558-9160	• Anti-viral reagents
American Type Culture Collection 301-881-2600 800-638-6597	• Cell lines • Viruses • Microorganisms • Recombinant DNA materials
Amgen Mr. Dennis McConnell 805-499-5725	• Cytokines • Growth factors • ELISA assays for erythropoetin (EPO), IL-6, and Granulocyte Colony Stimulating Factor (GCSF) • Human and murine DNA probes
Beckman Instruments Mr. Dennis Mooney 714-773-7603	• Monoclonal antibodies • HIV protein gene • Immunoassays
Becton Dickinson Mr. Philip Vorwald 408-954-2163	• Monoclonal antibodies • Flow cytometry equipment and supplies • Image analysis instruments

Boehringer Mannheim Biochemical
Dr. James Pease
800-428-5433

- RNA/DNA replication inhibitors

Cellular Products
Ms. Margaret Jones
716-842-6270

- Antibodies against HIV-1 and HTLV-I
- HIV-1 p24 ELISA and HTLV-I antigen ELISA
- Growth factors
- Immunofluorescence assay factors
- Antibody probe for HIV-1
- Western blots
- HIV-1 and HTLV-I antibody ELISAs

Collaborative Research
Ms. Laura Moore
800-343-2035
617-275-0004

- Growth factors
- Polyclonal antibodies against growth factors

Coulter Immunology
Mr. Brad Thornton
800-327-2729

- Monoclonal antibodies against leukocyte cell surface markers
- HIV p24 antigen microplate assay
- HIV p24 antigen neutralization kit
- HIV p24 antibody microplate assay test
- Western blot for HTLV-I and HIV-1

DuPont/NEN
Ms. Birgit Fleurent
617-350-9074
800-225-1572

- Monoclonal antibodies against HIV, HTLV-I, EBV, CMV, and HSV proteins
- p24 ELISA
- *env* 9 ELISA (recombinant)
- HIV antibody ELISA
- HTLV-I antibody ELISA
- HTLV-II ELISA
- EBV antibody ELISAs
- Western blot kits and strips
- Hybridization probes
- Recombinant viral proteins

Endogen
Mr. Philip Servidori
617-439-3250
FAX 617-439-0355

- Growth factors (natural cytokines, including interlukins)
- Antiserum
- Cytokine ELISAs including TNF, IL-1 and IFN
- Immunoassays
- Monoclonal and polyclonal antibodies against cytokines
- Cytokine immunoaffinity gel kit
- Custom reagents

Epitope
Mr. William Fleming
503-641-6115

- Western blot kit and monoclonals for HIV-1, HIV-2, and HTLV-I
- Western blot strips for HIV-1, HIV-2, and HTLV-I
- Monoclonal antibody kit control for HIV-1, HIV-2, and HTLV-I
- Reference laboratory service for confirmatory testing

GENETRAK
Dr. Jeffrey Klinger
508-872-3113

- Nucleic acid hybridization reagents and kits (HIV-1 + CMV)

Genzyme
Mr. Craig Powers
800-332-1042

- Growth factors
- Cytokine ELISAs
- Antibodies against cytokines
- HIV antibodies
- Glycoprotein remodeling agents

Gilead Sciences
Dr. Jeffrey Bird
415-574-3000

- Oligonucleotide analogues and conjugates

Imre
Dr. Harry Snyder
206-448-1000
206-448-1001

- ITP diagnostic test

Incstar
Mr. Fred Conway
800-328-1482

- RIAs for AZT, EPO and neopterin
- EIAs for β_2-microglobulin

International Enzymes
Mr. Paul J. Smith
619-728-5205

- Polyclonal antibodies
- HIV-1 antigen lysate
- HTLV-I antigen lysate
- HIV-2 lysate
- HTLV-III lysate
- p24
- gp41 on ELISA
- gp120 plate
- HIV $^{+}$ plasma

Life Technologies, Inc.

BRL
Ms. Karen Kryzwicki
301-670-8562

GIBCO
Customer Service
800-828-6686

- Cell and tissue culture media and reagents
- Balanced salt solutions
- Hybridoma reagents
- Animal sera and serum alternatives
- Restriction and modifying enzymes
- Electrophoresis apparatus and equipment
- Nucleic acid detection systems and reagents
- Transfection and transformation systems and reagents
- Immunodetection systems and reagents

Maryland Medical Laboratory, Inc.
Dr. Bill Meyer
301-247-9100 (Baltimore, MD)
800-638-1731 (MD)
800-368-2576 (USA)

- HIV-1 isolates (both USA and foreign)
- HIV-1 seropositive and seronegative specimans
- HIV-1 infected human mononuclear cells from peripheral blood (frozen in liquid N_2)

Medigenics, Inc.
Jack Kincaid or
Dr. Johnna Roberts
402-391-6944
FAX 402-391-7625

- Purified authentic HIV protease
- *lacZ* protease fusion protein

MicroGeneSys
Ms. Mary Lyons
800-541-8315

- HIV proteins
- Dot blot kits
- ELISA kits
- Animal antisera against HIV
- HIV-RT enzyme

Oncor
Ms. Pat Harrington
301-963-3500

- HIV-1 detection kit
- Southern blot reagents and kit
- HTLV-I and HTLV-III DNA and RNA probes for Southern blot analysis

Organon Technika
800-682-2666

- HIV-1 viral lysate
- HIV-1 ELISA kit
- HIV-1 Western blot kits

Pan-Data Systems
Ms. Christine Owens
301-294-2297
800-543-6059

- Growth factors
- Monoclonal antibodies to HTLV-I and HIV-1
- Specialized media
- HIV-1 and HTLV-I Western blot kits
- Fresh macrophage cultures
- Purified retroviruses

Raylo Chemicals
Mr. Matthew Colomb
403-620-2107

- Antiviral compounds
- Nucleosides

Scripps
Mr. David West
619-566-3505

- HIV-1 antigen (subclone of HUT 78)
- HTLV-I antigen (subclone of MT-2)
- CMV antigens
- HIV-1 positive plasma (psoralen inactivated)
- HTLV-I positive plasma (psoralen inactivated)
- HIV-1 in HUT 78
- Herpes Simplex Virus, Type 1 antigen
- Herpes Simplex Virus, Type 2 antigen
- β_2-microglobulin

Seikagaku America
Mr. Hiroyuki Morita
800-237-4512
301-424-0456

- Protein kinase inhibitors and activators
- Monoclonal antibodies

Synthetic Genetics
Mr. Ed Graham
619-587-0320
800-562-5544

- Custom Oligonucleotides

United States Biochemical Corporation
800-321-9322
FAX 216-464-5075

- Restriction enzymes
- Sequencing reagents
- Monoclonal antibodies

Vital Blood Products
Dr. Michael Flom
818-703-6000
FAX 818-703-6170

- HTLV-I antigen
- Hepatitis B surface antigens
- HIV $^{+}$ plasma
- Antibody to core antigen
- Antibody to C antigen
- Toxoplasmosis positive sera
- Hepatitis A IgM

Appendix G: Reagent Donation Form

Instructions:
Using the guidelines provided, please complete the appropriate data sheets to the best of your ability. The information you provide is crucial to use of reagents by recipients and reduces the necessity for contacting contributors.

Name:
Title:
Institution:
Department/Subdivision:
Full Address:

Telephone:( ) _____ FAX number:( ) _____

Reagent(s) donated:

1. Name: _____ Description: _____
2. Name: _____ Description: _____
3. Name: _____ Description: _____
4. Name: _____ Description: _____
5. Name: _____ Description: _____

To: AIDS Research and Reference Reagent Program
ERC BioServices Corporation
649A Lofstrand Lane
Rockville, MD 20850

I am donating the above reagent(s) to the AIDS Research and Reference Reagent Program. I have completed the appropriate data sheet(s) necessary to insure proper credit for donation and accurate usage of the reagent(s) and have included them with appropriate reprints.

Sincerely,

DATA SHEET GUIDELINES - CELL LINES

Name:

Provided:

Are there suggestions as to the amount of reagent distributed to the recipient?

Cell Type:

Please give a brief description including the parent cell line and cell lineage.

Medium For Propagation:

Freeze Medium:

Growth Characteristics:

Include viability, if known. At what concentration should cells be maintained? How often should they be passaged? What is the doubling time? How do cells grow (as a suspension, a monolayer, or semi-adherant)? Do cells grow singly or in clumps? Do cells require special consideration for thawing or propagation? Has the cell line been grown in other media? What are the essential requirements for growth?

Morphology:

Appearance of cells in culture.

Special Characteristics:

Special considerations for propagation could also be considered here. If this cell line is a variant on another cell line (e.g. HeLa CD4 is a variant of HeLa) please describe briefly how it was altered and how it differs from the parent cell line. Does the cell line secrete material? If so, please briefly describe what it secretes and how it was altered to cause secretion. What other special properties do these cells possess to make them of interest to the researcher (e.g. good for propagation of specific viral strains, etc.). Are the cells biohazardous, and if so, what precautions should be taken?

Sterility:

Have the cells been tested for mycoplasma? If so, what tests were used?

Contributor:

Are you the original source? If not, who is?

References:

The Repository does not have a ready source of journals. In addition to, or instead of providing citations, could you provide reprints? These help the Repository provide information to recipients who call with questions.

DATA SHEET - CELL LINES

Name:

Provided:

Cell Type:

Medium For Propagation:

Freeze Medium:

Growth Characteristics:

Morphology:

Special Characteristics:

Sterility:

Contributor:

References:

DATA SHEET GUIDELINES– VIRUS INFECTED CELL LINES

Name:

Provided:

Are there suggestions as to the amount of reagent distributed to the recipient?

Cell Type:

Please give a brief description including the parent cell line and cell lineage.

Propagation Medium:

Freeze Medium:

Growth Characteristics:

Include viability, if known. At what concentration should cells be maintained? How often should they be passaged? What is the doubling time? How do cells grow (as a suspension, as a monolayer, or semi-adherant)? Do cells grow singly or in clumps? Do cells require special consideration (e.g. do fresh cells have to be added due to cytopathic effects, special thawing instructions, etc.)? Has the cell line been grown in other media? What are the essential requirements for growth?

Morphology:

Appearance of cells in culture. Do cells form synctia?

Special Characteristics:

How was infected cell line obtained? Special considerations for propagation could also be addressed here. Does the cell line secrete material? To what extent does the cell line produce viral particles? What other characteristics make this cell line unique and/or of interest to the researcher (e.g. synthesizes defective viral particles, does the cell line synthesize more than just virus, does cell line express high levels of virus, etc.). As the cell line is biohazardous, what precautions should be observed?

Sterility:

Have the cells been tested for mycoplasma? If so, what tests were used?

Contributor:

Are you the original source? If not, who is?

References:

The Repository does not have a ready source of journals. In addition to, or instead of providing citations, could you provide reprints? These help the Repository provide information to recipients who call with questions.

DATA SHEET– VIRUS INFECTED CELL LINES

Name:

Provided:

Cell Type:

Propagation Medium:

Freeze Medium:

Growth Characteristics:

Morphology:

Special Characteristics:

Sterility:

Contributor:

References:

DATA SHEET GUIDELINES– VIRUS ISOLATES

Name:

Provided:

In what form does the virus come? Are there suggestions as to the amount of reagent distributed to the recipient?

Strain:

Titer:

In addition to the titer, please give tests used to determine it.

Original Source:

Please do not list the contributor from which the virus was obtained. Instead, list the original biological source.

Preparation:

Briefly describe the best conditions for virus propagation. In which cell lines does it grow best? What concentrations of cells and virus should be used to initiate infection? What medium should be used? Are there special considerations (e.g. are there cytopathic effects, etc.)?

Host of Choice:

Host Range:

Special Characteristics:

Describe original isolation and preparation of virus. What makes this virus of particular interest to the researcher?

Contributor:

Are you the original contributor? If not, who is?

References:

The Repository does not have a ready source of journals. In addition to, or instead of, providing citations, could you provide reprints? These help the Repository provide information to recipients who call with questions.

DATA SHEET– VIRUS ISOLATES

Name:

Provided:

Strain:

Titer:

Original Source:

Preparation:

Host of Choice:

Host Range:

Special Characteristics:

Contributor:

References:

DATA SHEET GUIDELINES – GENETIC CLONES

Name:

Provided:

In what form does the genetic clone come? Are there suggestions as to the amount of reagent distributed to the recipient?

Cloning Vector:

Bacterial Host:

Please give range, if known.

Cloning Site:

List the cloning site(s) on the vector into which the insert was placed, as well as the 5' to 3' orientation.

Source of Provirus:

Please give the original host and/or source as well as a brief description of how the provirus was prepared.

Description of Clone:

Please describe the genomic content of the insert and where the insert is physically oriented on the plasmid with respect to other special features. What is the genetic content of the insert (e.g. contains the first coding region of the tat gene in an open reading frame as well as a noncoding 5' end of the gag gene and a 3' noncoding end of the env gene). Is the insert located next to a special expression gene, such as CAT, β-galactosidase, etc.? Is expression driven by a specific promoter? If a map of the plasmid exists, could you include a copy (preferably with distances marked out in b.p.)? Has any of the clone been sequenced? If so, could you please include a copy?

Special Characteristics:

What makes this clone unique? Does it produce infectious virus particles? If so, in which cell lines and to what extent? What functional proteins does it encode/express? How does this protein differ from native protein? Does it only encode part of the protein? To what extent is the protein expressed? Under which conditions does the provirus give the best expression (i. e. in which cell lines does it work best?).

Contributor:

Are you the original source? If not, who is?

References:

The repository does not have a ready source of journals. In addition to, or instead of providing citations, could you provide reprints? These help the Repository provide information to recipients who call with questions.

DATA SHEET– GENETIC CLONES

Name:

Provided:

Cloning Vector:

Bacterial Host:

Cloning Site:

Source of Provirus:

Description of Clone:

Special Characteristics:

Contributor:

References:

DATA SHEET GUIDELINES – EXPRESSION SYSTEM

Name:

Provided:

In what form does the expression system come? Are there suggestions as to the amount of reagent distributed to the recipient?

Cloning Vector:

Cloning Site:

List the cloning site(s) on the vector into which the insert was placed, as well as the 5' to 3' orientation.

Cloning Strategy:

Briefly describe how the cloning vector and insert were prepared for recombination. Which restriction endonucleases were used to obtain the fragments? Where physically on the vector was the insert placed, and in what orientation? Were special techniques used to obtain a fragment with special characteristics (e.g. The 5' LTR of HIV-1 was recombined with the first coding exon of tat and the 3' LTR to give a sequence coding functional Tat (first coding exon))?

Host:

Please list the range of bacterial hosts.

Description of Clone:

Please describe the genomic content of the insert and where the insert is physically oriented on the plasmid with respect to other special features. What is the genetic content of the insert (e.g. contains the first coding region of the tat gene in an open reading frame as well as a noncoding 5' end of the gag gene and a 3' noncoding end of the env gene).Describe the promoter from which expression takes place and describe the procedure necessary for expression. If a map of the plasmid exists, could you include a copy (preferably with distances marked out in b.p.)? Has any of the clone been sequenced? If so, could you please include a copy?

Special Characteristics:

What makes this clone unique? What functional proteins does it encode/express? How does this protein differ from native protein? Does the protein have an authentic N- and C-terminus, or is it a run-off protein? Does it only encode part of the protein? To what extent is the protein expressed? Under which conditions does the plasmid give the best expression (i. e. in which cell lines does it work best?). What protocols have you developed to purify the protein? Please describe the assays you use to detect the protein.

Contributor:

Are you the original source? If not, who is?

References:

The repository does not have a ready source of journals. In addition to, or instead of providing citations, could you provide reprints? These help the Reopsitory provide information to recipients who call with questions.

DATA SHEET – EXPRESSION SYSTEM

Name:

Provided:

Cloning Vector:

Cloning Site:

Cloning Strategy:

Host:

Description of Clone:

Special Characteristics:

Contributor:

References:

DATA SHEET GUIDELINES – VIRAL PROTEINS

Reagent:

Provided:

In what form does the protein come? Are there suggestions as to the amount of reagent distributed to the recipient?

Molecular Weight:

Degree of Purity:

Please include the tests used to determine purity.

Activity:

Please include assays and assay conditions used to determine activity. How much protein is needed for a typical general experiment?

Production System:

Special Characteristics:

Give a brief description of how the protein was produced. If the protein was produced from a specific virus strain, please list this. Which purification methods were used? If the protein is lyophilized, in what buffer should it be resuspended and to what volume? If the protein is in solution, what is the buffer? Does the protein need preparation before use? If the protein needs to be resuspended in or diluted in a specific buffer before use, please include the composition and pH of the buffer. Is the protein especially suited to certain research applications? What other special characteristics does it posess? Is the protein glycosylated? If so, what was the host?

Contributor:

Are you the original source? If not, who is?

References:

The repository does not have a ready source of journals. In addition to, or instead of providing citations, could you provide reprints? These help the Repository provide information to recipients who call with questions.

DATA SHEET – VIRAL PROTEINS

Reagent:

Provided:

Molecular Weight:

Degree of Purity:

Activity:

Production System:

Special Characteristics:

Contributor:

References:

DATA SHEET GUIDELINES – MONOCLONAL ANTIBODIES

Name:

Provided:

In what form does the antibody come? Are there suggestions as to the amount of reagent distributed to the recipient?

Host:

Mouse, human, rabbit, goat, other (specify).

Isotype:

Please give both class and light chain type, if known.

Titer:

In addition to the data, please provide the tests used to determine titer. Please include the immunoglobulin concentration, if known. What dilutions do you use for specific experiments you perform with the antibody?

Special Characteristics:

What is the antibody directed against? Describe the antigens (e.g. synthetic peptide, purified protein, etc.) used to raise the antibody. Briefly describe the purification methods used to obtain the antibody. If the antibody is lyophilized, in what buffer should it be resuspended and to what volume? If the antibody is a supernatant, are there preservatives? Does the antibody show cross-reactivity? Have you prepared conjugate antibodies (e.g. alkaline phosphatase, biotinylated, enzyme labelled, fluorescence labelled)? Can you provide protocols for experiments you perform with these antibodies?

Contributor:

Are you the original source? If not, who is?

References:

The repository does not have a ready source of journals. In addition to, or instead of providing citations, could you provide reprints? These help the Repository provide information to recipients who call with questions.

DATA SHEET – MONOCLONAL ANTIBODIES

Name:

Provided:

Host:

Isotype:

Titer:

Special Characteristics:

Contributor:

References:

DATA SHEET GUIDELINES – POLYCLONAL ANTIBODIES

Name:

Provided:

In what form does the antibody come? Are there suggestions as to the amount of reagent distributed to the recipient?

Host:

Mouse, human, rabbit, goat, other (specify).

Titer:

In addition to the data, please provide the tests used to determine titer. Please include the immunoglobulin concentration, if known. What dilutions do you use for specific experiments you perform with the antibody?

Special Characteristics:

What is the antiserum directed against? Describe the antigen (e.g. synthetic peptide, purified protein, etc.) used to raise the antiserum. If the antiserum is lyophilized, in what buffer should it be resuspended and to what volume? If the antiserum is a supernatant, are there preservatives? Does the antiserum show cross-reactivity? Have you prepared conjugate antibodies (e.g. alkaline phosphatase, biotinylated, enzyme labelled, fluorescence labelled)? Can you provide protocols for experiments you perform with these antibodies?

Contributor:

Are you the original source? If not, who is?

References:

The repository does not have a ready source of journals. In addition to, or instead of providing citations, could you provide reprints? These help the Repository provide information to recipients who call with questions.

DATA SHEET – POLYCLONAL ANTIBODIES

Name:

Provided:

Host:

Titer:

Special Characteristics:

Contributor:

References:

Appendix H: Program Personnel

ERC BioServices Corporation

Susan Stern, PhD, Principal Investigator
Stephen Lindenfelser, Assistant Principal Investigator
Martha Matocha, PhD, Repository Scientist
David Pennock, Repository Scientist
Jacintha Stall, Repository Scientist
Darlene Williams, Administrative Assistant
Henry Groover, Systems Analyst

Advanced Biotechnologies, Inc. (Subcontractor for Expansion and Quality Control)

James Whitman, PhD, President
Kathleen Sybert, PhD, Head, Molecular Biology
Alison Demarest, Manager, Quality Assurance/Regulatory Affairs

Repository Operating Committee, Division of AIDS, NIAID

Edward Allen, Epidemiology Branch
James Cradock, Developmental Therapeutics Branch
Robert Eisinger, Treatment Research Branch
Stella Machado, Biostatistics Branch
Gregory Milman, Pathogenesis Branch
Linda Muul, Pathogenesis Branch
Nava Sarver, Developmental Therapeutics Branch
Alan Schultz, Vaccine Research and Development Branch

649A Lofstrand Lane, Rockville, MD 20850, (301) 340-0245, FAX: (301) 340-9245

Appendix I: NIAID Extramural Division of AIDS

The National Institute of Allergy and Infectious Diseases (NIAID) is the lead institute at the National Institutes of Health (NIH) for coordinating, conducting, and supporting AIDS research. In 1986, the Institute established the Acquired Immunodeficiency Syndrome Program, which is responsible for the management of research grants and contracts and other extramural activities supported by NIAID. In 1989, it was redesignated the Division of AIDS (DAIDS).

DAIDS supports research on many aspects of AIDS infection. The Treatment Research Program is involved with the clinical evaluation of therapeutic agents of potential use against infections by the human immunodeficiency virus (HIV). Within the Basic Research and Development Program, the Developmental Therapeutics Branch supports preclinical evaluation of therapeutics; the Vaccine Research and Development Branch funds preclinical and clinical studies of potential AIDS vaccines; the Pathogenesis Branch supports studies on the virology, molecular biology, and immunopathogenesis of HIV; and the Resources and Centers Branch supports centers of basic research and provides core research resources to assist other branches of the program. The Epidemiology Branch supports studies on the epidemiology and natural history of the disease. The Biostatistics Research Branch advises the other branches on study design and data collection and analysis as well as supporting research related to statistical methodology, mathematical modeling, and systems development for the collection and analyses of data related to studies of AIDS.

The Treatment Research Program is responsible for the support of research on the clinical development and evaluation of potentially effective therapies for HIV infection and related opportunistic infections. It is currently supporting the AIDS Clinical Trials Group, an extensive network of AIDS Clinical Evaluation Units located at medical centers around the United States. Investigators cooperate in the development of research protocols and conduct clinical trials of experimental therapies. The careful evaluation of potential therapies will provide the information needed to move experimental therapeutic agents into more widespread use.

Research on the preclinical development of therapies having potential for the treatment of HIV infections is supported by the Developmental Therapeutics Branch. This branch is responsible for studies aimed at identifying and developing strategies for treatment, including optimal approaches to therapy and novel methods of drug delivery, and evaluating the efficacy of therapeutic agents in cell and animal model systems. Multidisciplinary, multi-institutional National Cooperative Drug Discovery Groups have been established to facilitate the design, synthesis, and preclinical evaluation of treatment strategies for AIDS.

The Vaccine Research and Development Branch supports and coordinates the development and testing of vaccines to prevent HIV infection. Animal model systems are being developed for testing candidate vaccines. In 1988, NIAID's six Vaccine Evaluation Units began a Phase I trial of a recombinant vaccine consisting of the envelope protein, gp160, of the AIDS virus. A second experimental vaccine, using a vaccinia virus vector, is now undergoing testing in these units as well. In addition, the branch supports research resource activities to provide antisera, polypeptides, monoclonal antibodies, and viral pools to investigators engaged in research to develop an AIDS vaccine. National Cooperative Vaccine Development Groups were estab-

lished in 1988 to foster collaboration among academic research institutions, industry, and government by pooling their scientific talents and resources in vaccine development.

Research on the epidemiology of AIDS is supported by the Epidemiology Branch. In 1983, a Multicenter AIDS Cohort Study (MACS) was initiated at four centers to follow the natural history and epidemiology of HIV infection. Data from the MACS have yielded valuable information about the transmission of HIV infection and the role of certain cofactors in the pathogenesis of AIDS. The Epidemiology Branch has established a grant program for International Collaboration in AIDS Research. This program will link U.S. institutions to research units at overseas sites and will develop research centers of excellence in geographic areas with major health problems due to HIV infection.

The Pathogenesis Branch supports basic research directed toward understanding the complex pathogenesis of the AIDS virus. Support is provided for investigations into the biological properties, molecular biology, and host response to HIV infection to improve the basic understanding of the virus, to improve diagnostic and prognostic indicators, and to develop and improve methods of prevention and treatment. The branch also supports research and development of an animal model for HIV infection. Programs of Excellence in Basic Research on AIDS were established in 1988 to foster collaborative research initiatives. Together with initiatives in epidemiology, treatment, and vaccine development, pathogenesis studies are expected to serve as a solid basic research foundation upon which to build the knowledge necessary to prevent and cure AIDS.

Further information on DAIDS programs may be obtained by writing to:

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